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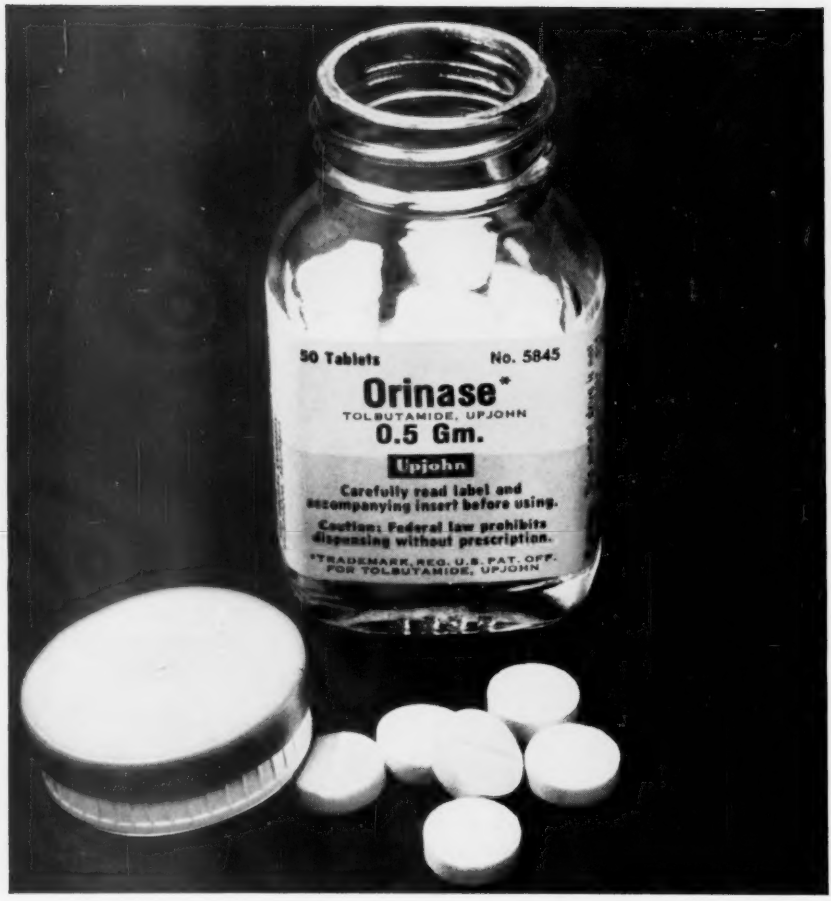

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THE YALE JOURNAL
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OBSERVATIONS ON THE CONTROL AND NATURAL HISTORY OF EPIDEMIC DIARRHEA OF INFANT MICE (EDIM)**

Previous work[5] has shown that epidemic diarrhea of infant mice (EDIM) is a virus-induced disease that can spread in mouse colonies by the airborne route. This fact made it imperative, under the ordinary conditions of animal care available at that time, to limit the incubation period of experimentally induced diarrhea to three days, since animals becoming ill after that time might have acquired the disease by cross contamination rather than from the inoculum. Thus the sensitivity of titrations, detection of virus, and neutralization tests was thought to be low, because it was not known if the incubation period following small inocula might not be longer than three days. This report will describe measures that allow control of airborne spread to the extent that incubation periods of seven or more days are routinely feasible. In addition, a few preliminary data pertinent to an understanding of this diarrhea will be presented.

EXPERIMENTAL

Establishment of a diarrhea-free colony of CFW mice. Because the diarrhea-free colony of mice that had been established for the experiments described previously[5] had to be abandoned, a new attempt was made to repeat this experience before the institution of the control measures described below.

Virgin male and female mice (CFW) five to six weeks old were obtained from Carworth Farms in September 1957. They were held until they were seven to eight weeks old and were then bred in regular open cages. The progeny were observed daily for external signs of spontaneous diarrhea, and in due course a few members of one litter were found to be diarrheal on the eighth day of life. The origin of the diarrhea was not immediately apparent. However, certain differences existed between the previous successful trial and the latter. First, in the former attempt the virgin animals

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* Research Associate in Pathology.

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were obtained in March rather than in September. Second, an animal caretaker was temporarily employed to help care for the second colony, whereas only the author and her assistants handled the mice in the first. Third, although the caretaker of the second colony had been asked to tend the mice before he had contact with other animals each day, it was later found that this was often impossible for him to do.

As a result of the single case of diarrhea, most of the colony (adults as well as infants) was destroyed, and all cages were sterilized in flowing steam on the day the disease was discovered. Nine 23-day-old females and two males were saved for breeding, and their progeny, born six weeks after the rest of the colony was destroyed but before the control measures were begun, were carefully observed for diarrheal signs. None became evident, and thus the present stock derived from these is considered to be diarrhea-free and has served as the source of the animals used for the experiments herein reported.

Measures for controlling experimental diarrhea. The special equipment used in the control of the airborne spread of EDIM virus consisted of "filter cages" and a bacteriological transfer hood.* Both were housed in the same animal room.

It was first thought that disposable cages might be constructed economically, but this did not prove practicable. Thus the present filter cages (Fig. 1) are made of galvanized iron cylinders provided with a flat, snugly fitting galvanized cover. A hole in the cover is just large enough to receive the stem of a water bottle. The main portion of the cylinder is fashioned of galvanized wire mesh (15 squares/in). The cages are 5½ inches high and 7 inches in diameter, thus presenting a floor area of about 38 square inches (Fig. 2). About the mesh area is tied a piece of Corning Fiberglas insulation ("Aerocor" PF 105) in such a way that the mesh is completely covered. If the mesh is too large, the adult mice chew the Fiberglas through the mesh and use it to line their nests. Large holes that communicate with the outside then result. Smaller cages were also tried (floor area of about 23 sq. in.), but they were not roomy enough for the female and litter.

While the animals are in such a cage, therefore, they are exposed only to filtered air. The insulation does not deter them from living in apparent comfort, and indeed they appear to be more contented in the darkness and seclusion offered by the filter material when rearing their families than

* The author wishes to express her thanks to Mr. Paul M. Lennard for his aid in designing the hood and for helpful suggestions in the filter cage construction.

they do in ordinary cages where they are more exposed to the outside world.

It is imperative, however, that the Fiberglas-mesh area be as large as possible (in the cages described, about 100 sq. in.) and that it form the sides of the cage rather than just the top, for example, of an animal jar. The latter arrangement was tried, using a single female and litter per jar, but there was apparently insufficient exchange between the inner and outer air, for after a few hours in such jars, the mice became agitated, did not nurse their young, and indulged in cannibalism.

The hood is depicted in Figure 3. It is made of Cheminert plastic and consists of an entry lock, hood proper, and exit lock. There are ultraviolet lights in both the hood proper and the exit lock. The latter is painted black. Two ports receive sleeves and gloves. At first neoprene sleeves and gloves were used, but they proved to be too clumsy. Therefore, surgical gloves were cemented to veterinary obstetrical sleeves and attached to the ports. These have been quite satisfactory.

Air enters the side of the hood proper through a Fiberglas filter and leaves by way of a permanent exhaust filter. The hood proper is always under negative pressure when in use. The reasons for this will be discussed below. The front panel of the hood proper is removable and, when the hood is in use, is sealed by means of a gasket of polyethylene tubing into which air is pumped to a pressure of 10 lbs./ sq. in.

Bedding, feed, and water. Cedar shavings were used throughout. Unsterilized tap water was given. Pathogen-free mouse and rat biscuits (Dietrich & Gambrill, Frederick, Maryland) supplemented with sunflower seeds, whole oats, and whole wheat were fed. No attempt was made to sterilize or pasteurize the grain, water, or bedding. Feed and shavings were kept in closed tins or covered pails, and only small lots of feed were stored at one time.

Breeding. Animals were bred in regular open cages in a room apart from the one in which the filter-caged animals were housed. Siblings were never mated. No more than four females were bred by one male. When the female was seen to be pregnant, she was placed alone in a filter cage regardless of the use to which her litter was to be put. Once animals were in filter cages, they were cared for only in the hood (see below). The young were born in the filter cage usually about one week after the pregnant female had been placed therein, and, if they were to be used as controls and breeders, the animals remained in that cage until the litter was 12 days old. At that time litter and dam were placed in a regular open cage, and the young eventually weaned and sexed. Breeding began when

the animals were six weeks of age, and their first litters were produced when the females were 9-11 weeks old. Thus, until they were 12 days old, mice that were to become breeders were not exposed to the common air of the animal room.

Results of the breeding program. From January to September 1958, 328 litters were born and handled in the manner just described. Of these, 179 have been fed various materials to test for the presence of EDIM virus, 140 were used as controls and for breeding, and 9 were discarded for a variety of reasons (cannibalism, death of parturient female, etc.). In none of the 140 litters used as controls was external evidence of diarrhea seen at any time. A few members of some of these litters were autopsied at various times, and in none of them was there internal evidence of diarrhea.

Extensive records were kept on 62 control litters to ascertain the numbers of mice still alive and well at 18 days of age. Such records were not available for all litters, since, in many cases, only as many males were saved as were needed to carry on the breeding program. The total number of infants in the 62 litters was 541, and the number alive at 18 days of age was 526 (97 per cent). The average litter size of primiparae was 8.5. Thirteen of the 62 families were re-bred. The average size of their first litters was nine, and the average size of their second litters was 11.5 infants.

Handling of animals in filter cages and transfer hood. Negative pressure in the hood proper was desirable for the following reasons. The assumption had to be made that EDIM virus was not present in the common air of the animal room in which the hood was housed, for all diarrheal animals were confined to filter cages. Since animals in filter cages were handled only in the hood, it was important that dust raised when any filter cage was opened should be immediately filtered and exhausted so that it would not contaminate the next cage of mice examined. Whether this is accomplished by positive or negative pressure is immaterial, but, if positive pressure were to be used in this instance, then contaminated air would be forced into the entry and exit locks when their inner doors were opened, and this contaminated air under positive pressure would then enter the room air when the outer doors of the locks were opened. Ultraviolet light alone could not be depended upon to de-contaminate outgoing air.

Mice were inspected each day before the examiners had handled other animals or virus preparations. The cages entered the hood via the entry lock one at a time (Fig. 3). In the hood proper, the cage was opened and the animals picked up for inspection with forceps that were otherwise kept in 95 per cent ethanol. Food was added to the cage, if needed, from

FIG. 1. Filter cages. The cage on the left is shown without filter material; that on the right is the complete unit. The broad stopper of the water bottle does not allow the bottle to tip. In the foreground is an inverted cover showing the apron that fits snugly inside the top of the cage.

FIG. 2. Litter and dam in filter cage. The litter is 11 days old and contains 14 infants. There is ample room for all.

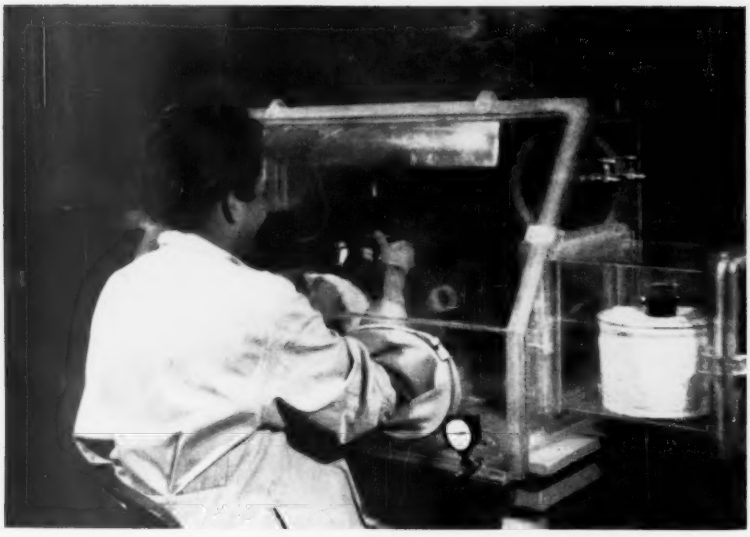

FIG. 3. Bacteriological transfer hood. A litter is being examined in the hood proper and another cage is seen in the entry lock. The exit lock is at the left and the exhaust filter box is on top of the hood proper. The exhaust fan is above the filter box and is not shown.

FIG. 4. Filter cages arranged on rack. These contain either infected or control litters. They can actually be placed closer together than depicted here without the occurrence of cross-contamination by EDIM virus.

individual food containers (3 oz. Dixie cups) that were passed into the hood. If water was needed, a freshly filled bottle was exchanged for the old; fresh water was never added to the old bottle. If the cage required changing, a clean one was passed into the hood, animals were transferred with forceps, and both cages were closed and placed in the exit lock in which the ultraviolet light was always on when the hood was being used. All materials leaving the hood remained in the exit lock for about one minute before they were removed; dirty cages and used water bottles were placed immediately into containers for sterilization. After passing through flowing steam for 45 minutes, the filter material was removed from the cages, the latter cleaned, washed, and dried, and new filter material tied around. Water bottles were thoroughly scrubbed before further use.

After use the hood proper was swabbed with alcohol, and, with the ultraviolet light on and exhaust fan still operating, was allowed to stand for at least 30 minutes.

When animals were to be fed or inoculated with virus, it was done after the examination of all other filter-caged animals and after the hood had had its preliminary cleaning; usually there was a period of at least three hours between the examination of animals and the infecting of new ones.

Method of feeding virus; diagnosis of diarrhea. These were the same as reported previously[5] except that the mice were fed in the transfer hood. After each litter was fed, the gloved hands were thoroughly swabbed with 95 per cent ethanol as was the floor of the hood proper. For autopsy, mice were taken to another room. The dams of litters that were autopsied were destroyed whether or not their young were diarrheal at autopsy.

Efficacy of the control measures. In an attempt to demonstrate the practicability of the control method just described, 24 litters of mice five to eight days old were employed. Seven litters were fed 10^4 ID_{50} of EDIM virus in the form of an intestinal (intestines and contents) filtrate. (The dosage was determined by subsequent titration of a previously frozen (-70° C.) aliquot of the filtrate). Seven litters were exposed in the hood; i.e., after each of the "fed" litters was given virus, another litter was passed into the hood, the cage opened, and the animals handled in identical fashion except that no virus was fed. Ten litters remained as uninoculated, unexposed controls. None of the animals showed external evidence of diarrhea at the start of the experiment. When the cages were placed on the rack, those containing litters that had been fed virus were purposely put in close proximity to the control and exposed litters (Fig. 4).

Mice were examined daily in the hood. At various times after feeding, mice of all groups were autopsied for internal evidence of diarrhea. The

results are seen in Table 1. Diarrhea, on either external or internal examination, began on the third day after the start of the experiment and was confined in all cases only to those mice that had been fed the agent (F, in Table 1). None of the exposed (P) or control (C) groups manifested either external or autopsy evidence of diarrhea. Data for litter No. 1 in Table 1 re-emphasize what had been noted previously,[6] viz. that autopsy is essential to establish a firm diagnosis of diarrhea, in other words, that lack of external soiling does not necessarily signify absence of the disease.

Because the diarrhea in this experiment was severe with external soiling, particularly in the younger animals, and because there was ample time (cf. Table 4 and accompanying text) for the exposed and control animals to contract the disease by cross contamination, it appeared reasonable to assume that future positive findings would be the result of virus-containing inoculum only.

Comparison of allowable incubation periods (three and seven to ten days). On the basis of the results just described, a considerable increase in incubation period over the three days previously used[5] could be allowed. It was expected that this might enhance the sensitivity of viral detection. For convenience of feeding mice, three-day-old animals were used, because it is easier to instill material into their mouths than it is in younger animals. A maximum period of seven days was given, for after the mice are ten days old, the colonic contents of normal mice began to change color and consistency making borderline cases of diarrhea difficult to assess at autopsy. With this scheme a single intestinal filtrate was titrated and the final titer determined at autopsy three and seven days after feeding.

With the size of breeding colony available, there were not enough litters to perform a complete titration on a single day. Therefore filtrates and suspensions to be tested or titrated were frozen at -70° C. in small amounts in sealed ampules. The required dilution(s) was then made from one of these aliquots the day it was to be fed. Once thawed, the aliquots were not re-used for titration data.

The results are given in Table 2. It is clear that the longer incubation period permits detection of 100 to 1000 times less virus than the three-day period. Other comparisons have also indicated increased sensitivity of the seven-day period (see Table 4).

The data in Table 2 do not, of course, demonstrate that an incubation period longer than seven days might not result in even greater sensitivity. Therefore, a single suspension of infant mouse liver containing about $10^{3.5}$ ID_{50} per oral dose for three-day-old mice with a seven-day incubation period was re-titrated at 10^{-3} and 10^{-4} dilutions in mice 0 - 3 days of age.

TABLE 1. DEMONSTRATION OF CONTROL OF AIRBORNE SPREAD OF EPIDEMIC DIARRHEA OF INFANT MICE (EDIM) BY MEANS OF FILTER CAGES AND BACTERIOLOGIC TRANSFER HOOD

Litter number	*Age when fed (days)*	*Number of infants*	*Treatment*	*Days after feeding EDIM virus*												
				1 E	2 E	3 E	3 A	4 E	5 E	5 A	6 E	7 E	7 A	8 E	9 E	9 A
1	8	5	F	—	—	—	1/1*	—	—	1/1	—	—	1/1	—	—	0/2
2	8	6	F	—	—	—	1/1	—	+	1/1	—	—	1/1	—	—	0/3
3	8	5	P	—	—	—	nd	—	—	0/1	—	—	0/1	—	—	nd
4	8	4	P	—	—	—	nd	—	—	0/1	—	—	0/1	—	—	nd
5	8	6	C	—	—	—	0/2	—	—	nd	—	—	0/1	—	—	nd
6	8	7	C	—	—	—	0/2	—	—	nd	—	—	0/1	—	—	nd
7	7	8	F	—	—	+	2/2	+	+	nd	+	+	1/1	+	—	0/5
8	7	4	F	—	—	+	nd	+	+	nd	+	—	1/1	+	—	0/3
9	7	7	P	—	—	—	0/2	—	—	0/1	—	—	0/1	—	—	nd
10	7	8	P	—	—	—	0/2	—	—	0/1	—	—	0/1	—	—	nd
11	7	5	C	—	—	—	0/1	—	—	0/1	—	—	0/1	—	—	nd
12	7	8	C	—	—	—	0/2	—	—	0/1	—	—	0/1	—	—	nd
13	6	7	F	—	—	+	nd	+	+	nd	+	+	1/1	+	—	0/6
14	6	3	F	—	—	+	nd	—	+	nd	—	—	1/1	—	—	0/2
15	6	8	P	—	—	—	0/2	—	—	0/2	—	—	0/1	—	—	nd
16	6	3	P	—	—	—	nd	—	—	0/1	—	—	0/2			
17	6	5	C	—	—	—	0/1	—	—	0/1	—	—	0/1	—	—	nd
18	6	7	C	—	—	—	0/2	—	—	0/1	—	—	0/1	—	—	nd
19	6	3	C	—	—	—	nd	—	—	0/1	—	—	nd	—	—	nd
20	6	4	C	—	—	—	0/1	—	—	nd	—	—	0/1	—	—	nd
21	5	6	F	—	—	+	nd	—	+	2/2	—	—	1/1	+	—	0/3
22	5	9	P	—	—	—	0/2	—	—	0/1	—	—	0/1	—	—	0/5
23	5	8	C	—	—	—	0/2	—	—	0/1	—	—	0/1	—	—	nd
24	5	7	C	—	—	—	0/2	—	—	0/1	—	—	0/1	—	—	nd

E = external diagnosis
A = autopsy diagnosis
F = fed 10^4 ID_{50} EDIM virus
P = passed through hood (see text)
C = control (see text)

— = no diarrhea upon external examination
+ = diarrhea present upon external examination
nd = not done
* In the fractions, the numerator indicates the number of diarrheal mice, the denominator, the number of mice autopsied.

All litters were autopsied when they were 10 days old, thus giving incubation periods of 7-10 days.

The results are presented in Table 3. It is evident that there is no advantage in using younger mice together with a longer incubation period; for, despite the fact that only one litter was used for two of the 10^{-3} determinations, the titer of the material tested is essentially the same in all groups of animals.

It must be noted, however, that the single litter that became diarrheal after feeding on the day of birth (0 days old) was the most miserable,

TABLE 2. DEMONSTRATION OF THE INCREASE IN SENSITIVITY OF EDIM VIRUS DETECTION USING THE 7-DAY INCUBATION PERIOD

Virus dilution fed	*Allowable incubation period*	
	3 days	*7 days*
10^{-4}	**2/2** (12/12, 8/8)*	**1/1** (8/8)
10^{-5}	**1/2** (5/6, 0/5)	**2/2** (9/9, 7/7)
10^{-6}	**0/2** (0/7, 0/9)	**2/2** (8/8, 8/8)
10^{-7}	**0/2** (0/8, 0/10)	**2/2** (6/8, 10/10)
10^{-8}		**0/2** (0/8, 0/10)
ID_{50}/litter inoculum	10^{5}	$10^{7.5}$
ID_{50}/ml	$10^{6.3}$	$10^{8.8}$

* In the first fraction, the numerator represents the number of diarrheal litters at autopsy, the denominator, the number of litters fed. In the fractions in parentheses, the numerator indicates the number of diarrheal infants found, and the denominator, the number of infants in each litter.

stunted, decrepit litter of all those reported in this paper. It is conceivable that mice infected at birth are the most severely affected by this disease in colonies where it is endemic.

Several assumptions, which appear reasonable at present, are made in evaluating the titration studies: first, that the mice used are uniformly susceptible (barring the usual biological variation); second, that once diarrhea has begun in infants it does not cease until the 14th-16th day of life[5]; and, third, that no EDIM virus is present in the breeding animals. Since the entire litter must be considered the experimental unit, it is perhaps best to think of the amount of inoculum per litter rather than per mouse. Calculations indicate that each mouse consumes about 0.008 ml. of inoculum; however, feeding India ink (see below) showed that the inoculum was not only in the infants but was also on them and on their dam. Thus, the precise amount that is first swallowed by the mice is not known,

but it is considered to average 0.05 ml. per litter. Therefore ID_{50}'s are calculated for 1 ml. by multiplying the ID_{50}/litter inoculum by 20. Titration data at present are probably more accurate than ± 1.0 log and perhaps as accurate as ± 0.5 log, but a statistical analysis has not yet been made.

At, or near, the end point of a titration, where an entire litter may not be diarrheal at autopsy, calculations based on number of mice rather than on number of litters have not affected the titer or have increased or decreased it at most 0.5 log. This difference is not considered significant at

TABLE 3. RESULTS OF FEEDING EDIM VIRUS TO INFANT MICE AT DIFFERENT AGES WITH VARIOUS ALLOWABLE INCUBATION PERIODS

Virus dilution fed	*Age at feeding (days)*	*Incubation period (days)*	*Results*
10^{-3}	3	7	**1/1** (9/9)*
	2	8	**1/2** (1/8, 0/10)
	1	9	**1/1** (9/9)
	0	10	**1/2** (7/7, 0/10)
10^{-4}	3	7	**0/2** (0/10, 0/5)
	2	8	**0/2** (0/8, 0/9)
	1	9	**0/2** (0/8, 0/10)
	0	10	**0/2** (0/10, 0/11)

* See legend, Table 2.

present. However, titers obtained by such a calculation are deemed false, since there is no way of knowing how many of the diarrheal mice in a particular litter acquired the disease from the inoculum and how many from their siblings.

Detection of EDIM virus by other means. Thus far no other test system except oral inoculation of infant mice has been successful for detecting the presence of EDIM virus. Tissue cultures [monkey kidney epithelium, minced mouse embryo in Maitland-type cultures, L strain fibroblasts, HeLa, human intestine (Henle), human conjunctiva, MAF (Microbiological Associates fibroblasts)] have all given negative results as far as a cytopathic effect is concerned. Although chick embryos do not appear to be affected when the chorioallantois is inoculated, the agent survives there for at least five days, and it is conceivable that it might be adapted to the embryonated hen's egg. Red cells (final concentration 0.125 per

cent at pH 7.2 in 0.15 M phosphate buffer) of the following species are not specifically agglutinated or hemolyzed at room temperature by 10^8 ID_{50} per ml.: guinea pig, adult and embryonic chicken, adult and infant mouse, rabbit, hamster, human O, sheep.[8]

The subcutaneous and intraperitoneal routes have not been used routinely in infant mice, since, in animals of this size, some of the inoculum has a tendency to leak back through the needle track and may therefore find its way to the oral cavity; at least, oral intake of some exuded inoculum cannot be ruled out. It appears that infant mice can be infected by the above routes, although they seem to be less sensitive than the oral. It is possible that the subcutaneous and intraperitoneal routes are indeed highly insensitive, and that mice thus inoculated become diarrheal only after some of the exuded inoculum reaches the oral cavity. This matter has not been investigated further.

Results of EDIM virus detection using the control measures. Although the numbers of litters available for testing were limited, some preliminary and as yet incomplete information, in addition to that regarding control of cross-contamination, has been gathered. The data are presented at this time to demonstrate the kind of information that can be obtained using the control measures and to indicate the futility of controlling epidemic diarrhea of infant mice by destroying only diarrheal young.

Fate of ingested EDIM virus in infant mice. Two litters of three-day-old mice were each fed 10^4 ID_{50} of EDIM virus in the form of intestinal filtrate. At various times thereafter (3, 22, 30, 72 hours, and 6 days) two or more mice from each litter were sacrificed. Blood was taken by cardiac puncture after swabbing the skin thoroughly with a staturated solution of iodine in 95 per cent ethanol followed by 95 per cent ethanol rinse. It was defibrinated by shaking in a test tube that contained a small amount of sterile alundum; 0.1-0.2 ml. were obtained from each animal. Organs were removed thereafter as carefully as possible in order to avoid contamination of one by the other. Each type of harvested material was pooled and frozen at -20° C. until used. Organs (except the alimentary tract) were prepared as 10 per cent suspensions in broth. Coarse particles were removed by slow centrifugation. The alimentary tract and contents were similarly prepared except that Seitz filtrates of the separate portions were made. Each material was fed to one or, in most cases, two litters. The results are seen in Table 4.

On the assumption that no case of diarrhea was the result of cross-contamination, Table 4 shows that three hours after a large amount of virus was fed, it could be recovered from the stomach, small intestine, and large

TABLE 4. DISTRIBUTION OF EDIM VIRUS IN INFANT MICE AT VARIOUS TIMES AFTER FEEDING 10^4 ID_{50}

Material and dilution tested		*Time after feeding when materials were collected*								
		3 hours		*22 hours*		*30 hours*			*72 hours*	*6 days*
		7-day†	*3-day*	*7-day*	*3-day*	*7-day*	*3-day*		*3-day*	*7-day*
Blood	10^0	**0/2** (0/10, 0/8)*		**1/1** (10/10)		**2/2** (10/10, 9/9)				**2/2** (10/10, 11/11)
	10^{-1}					**2/2** (9/9, 9/9)				
	10^{-2}					**1/2** (0/6, 12/12)				
Brain	10^{-1}			**0/2** (0/5, 0/9)		**1/2** (6/8, 0/8)			**0/1** (0/6)	
Liver	10^{-1}	**0/2** (0/10, 0/8)	**0/1** (0/10)	**2/2** (11/11, 8/8)	**0/1** (0/5)		**1/1** (3/8)		**1/1** (7/7)	
	10^{-2}								**1/1** (6/6)	**2/2** (8/8, 5/5)
	10^{-4}								**0/1** (0/7)	**0/2** (0/9, 0/5)
	10^{-6}									**0/2** (0/6, 0/8)
Spleen	10^{-1}	**0/2** (0/10, 0/8)	**0/1** (0/11)	**2/2** (10/10, 11/11)	**0/1** (0/8)		**1/1** (4/7)		**1/1** (7/7)	
Kidney	10^{-1}	**0/2** (0/9, 0/12)	**0/2** (0/3, 0/3)	**0/1** (0/8)	**0/1** (0/11)	**2/2** (6/6, 7/7)			**1/1** (2/6)	
Stomach	10^{-1}	**2/2** (10/10, 9/9)	**0/2** (0/7, 0/8)	**2/2** (13/13, 9/9)			**1/1** (4/9)			
Sml intestine	10^{-1}	**1/2** (0/9, 5/7)	**0/1** (0/9)		**1/1** (7/7)		**1/1** (10/10)		**2/2** (8/8, 9/9)	**2/2** (8/8, 11/11)
Lrg intestine	10^{-1}	**2/2** (6/7, 8/8)	**0/1** (0/10)		**1/1** (8/8)		**1/1** (10/10)		**2/2** (5/5, 6/6)	**2/2** (7/7, 8/8)
Lungs	10^{-1}	**0/2** (0/6, 0/4)		**2/2** (10/10, 9/9)						
Bladder and urine	10^{-1}			**0/1** (0/12)		**1/2** (1/9, 0/3)				

Diarrhea begins about 40 hours

* See legend, Table 2.

† 7-day indicates that test mice were autopsied and diagnosed 7 days after feeding.
3-day indicates that test mice were autopsied and diagnosed 3 days after feeding.

intestine. To test the validity of this apparently rapid transport through the alimentary tract, a litter of eight three-day-old animals was fed India ink in the same amount as the viral inoculum. At the end of three hours the mice were sacrificed. In all of the eight animals most of the ink was found in the colon three - eight mm. from the anus. Small amounts were also seen in the cecum and terminal portion of the ileum, but none was grossly visible in the jejunum, duodenum, or stomach.

At the same time (three hours) virus was not demonstrable in the blood or other organs tested. It is noteworthy that the negative findings in the lungs at this time indicate that the inoculum was probably not aspirated.

Twenty-two hours after feeding, virus was found in blood and in all of the organs tested except brain, kidney, and bladder and urine. At 30 hours the kidneys became positive, and small amounts could be detected in brain and bladder and urine as well. In view of the viremia ($10^{3.3}$ ID_{50}/ml.), it would be surprising if this were not so at this time.

Despite the widespread distribution of EDIM virus in the mice, diarrhea, as evidenced by external signs, did not begin until about the 40th hour after feeding. Mice autopsied at 30 hours showed no internal indication of diarrhea. Virus was still present six days after feeding in blood, liver, and intestines. Other organs were not tested at that time.

In comparing the three-day with the seven-day incubation period for testing the presence of virus, the data, especially for stomach and small and large intestine at 3 hours and spleen and liver at 22 hours, corroborate the finding that the longer incubation period lends more sensitivity to virus detection.

Fate of ingested virus in previously nondiarrheal nursing females. It was previously stated[5] that one of the difficulties in diagnosing diarrhea on the basis of external soiling of infant mice rests in the fact that the nursing female is generally compulsive about cleaning her young. She must, therefore, ingest enormous amounts of virus during the course of the infection in her litter.

In order to ascertain the fate of such ingested virus, materials were taken from a few groups of females at the time when their diarrheal litters were sacrificed. The organs and blood were handled in the same way as those of their infants. Fecal pellets were prepared as a 10 per cent suspension in broth and the suspension filtered after preliminary slow centrifugation. The results are seen in Table 4. It is evident that the previously uninfected dam is capable of eliminating rather large amounts of virus in her feces (groups B and C) at least while she is with her diarrheal litter. In addition, her liver, spleen, and blood can also harbor

virus, indicating that she undergoes a generalized infection similar to that in her infants; but she appears to remain well while it is in progress.

DISCUSSION

It is noteworthy that repetition of the original attempt to establish a diarrhea-free colony of mice did not meet with success, but that a third trial using a small nucleus of mice derived from those used in the second attempt did result in an apparently diarrhea-free colony. The source of the infection that caused the second failure is unknown. It might have come from the mice themselves, since the animals were obtained from a diarrheal colony and might thus have been carriers. On the other hand, their carrier state is not known and it is quite as conceivable that infection was introduced from some other source. It is not known whether the spontaneous diarrhea that did occur was identical with the one now under study, nor is it known why the disease has not occurred in the progeny of that small nucleus of animals that began the present colony.

In feeding, handling, and caring for filter-caged animals in the transfer hood, the decision was made to use the simplest possible procedure that might insure confinement of EDIM virus to the cage and litter into which it was introduced. It is possible that the measures adopted are more complicated than necessary for this control; however, the use of a more relaxed technique for experimental purposes is not contemplated. How these measures might be modified for use in a commercial colony or in other large breeding establishments is not yet known. A minor possibility for spread of diarrhea virus rests in the fact that the infants manifest viremia for a rather long period of time; thus blood-sucking arthropods might be vectors of the agent. Fomites are obvious means of dissemination, especially in the case of an agent as heat resistant[5] as EDIM virus seems to be. If animals infected at birth are indeed most severely affected by the disease, perhaps abandonment of promiscuous breeding and nursing as ordinarily carried on in mouse-producing establishments and replacement of this practice by some other method whereby newborn mice are sheltered from the EDIM virus might be instituted. Whether or not this would be economically feasible is another question, however. Obviously more work will have to be done on the practical control or abolition of the disease.

Although the data cited above indicate that the measures instituted for control of diarrhea seem to have been successful, the critical experiment is lacking, viz., the continued rapid blind passage of intestinal filtrate from normal control animals to new mice. This procedure was considered, but it was decided that spontaneous diarrhea would not be prevented by this

means and that, at any rate, the breeding program as it was conducted would eventually allow the expression of such a "break." In addition, continued blind passage might even prove to be a waste of valuable animals. For the present, therefore, it is not unreasonable to assume that if diarrhea occurs in a litter of mice after feeding materials to test for the presence of virus, the disease is due to the fact that the inoculum contains virus and not because cross-contamination from neighboring diarrheal animals has occurred. Should diarrhea be found in control and breeding animals in the future, it will probably be because of the method of handling. The bacteriological transfer hood is not, of course, foolproof, for it is possible that after a diarrheal family passes through it followed by a normal litter, the normal one may receive enough airborne inoculum to cause infection. Actually, the probability of this occurring is quite remote since dust particles are immediately exhausted from the hood proper, and although room air does find its way into the entry lock when a cage is placed therein, that air too is immediately exhausted from the hood proper when the inner door of the entry lock is opened and before the cage is opened. Another possible cause for future failure rests in the results presented in Table 5, i.e., that adults that were nondiarrheal in infancy are perfectly capable of becoming infected with EDIM virus and eliminating it in their feces without themselves appearing ill in any way. Thus in the breeding cages, which are ordinary open mouse boxes, the adult animals may contract the infection if the agent is found in the milieu. This is not the only recorded instance in which dams can become infected from their sucklings, for Fellowes and Dimopoullos[4] report that this is the case for the Indiana strain of vesicular stomatitis virus.

This, of course, brings up the problem of how a previously nondiarrheal colony of mice becomes diarrheal in the first place. What is the carrier species? Or is the virus brought into a clean colony by fomites or ectoparasites only? Once a colony is infected, there is no question of the possible factors that can keep the epidemic going, but what starts the events that lead to the epidemic? At the moment the question is open, but rather enticing results are being obtained to indicate that humans, and perhaps other animals, may have neutralizing antibodies against this or an immunologically related agent.[6] Thus an attempt is being made to find the virus in other hosts besides the mouse.

It is not satisfactory that an entire litter of mice must be considered an experimental unit, nor that at present it is impossible to use more than two such units per dilution of a titration, for this makes end-points fairly inaccurate and diffuse. Allowing incubation periods longer than seven days does not appreciably increase the sensitivity of virus detection, but

increasing the number of litters used for each determination would probably increase the accuracy of titration end-points. In this regard, it would be more than desirable to be able to use each infant mouse as a unit, but to put such a scheme into operation would be prohibitively costly and might

TABLE 5. DISTRIBUTION OF EDIM VIRUS IN PREVIOUSLY NONDIARRHEAL DAMS WHEN NURSING DIARRHEAL LITTERS

Nursing female	*ID_{50} virus fed to litter*	*Dam sacrificed; days after litter fed*	*Materials tested*	*Dilution tested*	*Results**
A (1)**	10^{9}	7	Blood	10^{0}	**2/2** (3/6, 2/14)*
			Liver	$10^{-0.3}$	**2/2** (2/7, 2/9)
			Spleen	$10^{-0.8}$	**2/2** (8/8, 8/8)
				10^{-2}	**0/2** (0/8, 0/9)
			Feces†	10^{-1}	**1/2** (2/11, 0/13)
				10^{-3}	**0/2** (0/8, 0/10)
				10^{-5}	**0/2** (0/7, 0/10)
B (3)	$10^{6.3}$	7	Blood	10^{0}	**0/3** (0/9, 0/9, 0/7)
			Liver	$10^{-0.3}$	**2/2** (5/5, 10/10)
			Spleen	$10^{-0.8}$	**2/2** (7/7, 11/11)
			Feces†	10^{-1}	**2/2** (10/10, 9/9)
				10^{-4}	**1/2** (3/5, 0/11)
C (1)	10^{4}	4	Liver	10^{-1}	**0/2** (0/2, 0/9)
			Feces†	10^{-1}	**2/2** (10/10, 3/3)
				10^{-3}	**2/2** (10/10, 7/7)
				10^{-5}	**1/2** (11/11, 0/9)
D (2)	10^{4}	6	Liver	$10^{-0.3}$	**2/2** (9/9, 8/8)
			Feces†	10^{-1}	**2/2** (6/6, 6/13)
				10^{-4}	**0/2** (0/9, 0/9)

* See legend for Table 2.
** The numbers in parentheses indicate the number of dams in each group.
† Filtrate.

even fail. It is furthermore unfortunate that there is currently no other sensitive means of detecting EDIM virus. Perhaps another will never be found, but it would be most useful to adapt the agent to another host so that at least neutralization tests might be performed in that host (*e.g.* the embryonated hen's egg, as suggested above).

It is a continually fascinating fact that EDIM virus should be so widespread in infant mice both before and during diarrhea and yet produce

no marked microscopic evidence of infection.[1, 5, 7–9] It will be most interesting to see what the chronology of virus spread is when a small dose (10^0 to 10^1 ID_{50}) is fed. With 10^4 ID_{50} (Table 4) a great deal is obviously going on between 3 and 22 hours after feeding, but from the data at hand the site of primary multiplication is not evident. Additional work, using infectivity tests, fluorescent antibody technique (Coons), electron microscopy, and histochemical methods will doubtless elucidate details of pathogenesis in infants previously uninfected and previously infected adults.

It would be amiss, in a report dealing with the control of epidemic diarrhea of infant mice, not to say a few words regarding the theoretic possibilities for producing a protective vaccine in mice. Rather large pieces of information are lacking, the acquisition of which might enable a reasonably intelligent discussion of the subject, for the data obtained from experiments with diarrhea-free mice are not automatically valid for a diarrheal colony, i.e., one in which an epidemic is almost always smoldering and often erupting. In such a diarrheal colony the observation has been repeatedly made and reported[1–8, 7, 10] that the first litter of a female is not the only one that is susceptible to the disease. Indeed, second, third, and fourth litters (perhaps later ones as well, if autopsy evidence were to be used) are equally subject to diarrhea. This bodes ill for successful transfer of either natural or artificially induced immunity to the young via placenta and/or milk. The subject requires additional study.

Many questions relative to the natural and experimental disease are still unanswered; it is possible, however, that future data obtained with the aid of the control techniques described herein will resolve some of them.

Addendum. Identification of a bacterium reported previously[5] to be associated with epidemic diarrhea of infant mice has been accomplished. The organism is *Clostridium tertium,* mistaken originally for a *Bacillus* because it can be cultured aerobically. Since this is a common inhabitant of the gastrointestinal tract, its predominance reported at that time in certain diarrheal mice might be ascribed to opportunistic multiplication in deranged intestines. No significance in the etiology of EDIM can be attached to these organisms, except that they, as well as others, may contribute secondarily to the severity of the disease. In this regard, it would be most interesting to study experimental epidemic diarrhea of infant mice in germfree mice.

SUMMARY

1. The establishment of a colony of CFW mice free of epidemic diarrhea of infant mice (EDIM) is described.

2. Measures and techniques that have been devised to control the airborne cross-infection that plagues work on this disease under ordinary conditions of animal care, and evidence for successful control, are presented.

3. Data show that a seven-day allowable incubation period is 100-1000 times more sensitive in detecting EDIM virus than is a three-day period. Increasing the incubation period up to ten days leads to no additional increase in sensitivity.

4. Using the control measures and allowing a seven-day incubation period for testing, the fate of ingested EDIM virus was studied in infant mice and in their previously nondiarrheal nursing dams.

5. Factors pertinent to an understanding of the natural history of the infection in infant mice are discussed.

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THE RESPONSE OF HYPO- AND HYPERTHYROID IMMATURE FEMALE MICE TO GONADOTROPHIN (FSH)†

The level of thyroid function influences the responses of the ovaries to anterior pituitary gonadotrophic preparations and also ovarian function.[2, 13, 14] The nature and extent of ovarian responses of hypothyroid rats[2, 5, 6, 9, 13, 14] and mice[6, 9] to extrinsic gonadotrophins have been studied extensively. The thyroid level also may influence the threshold of responsiveness or quantitatively limit the response of end organs such as the ovarian response to endogenous gonadotrophins[15] or the vaginal response to estrogen.[7, 10, 14, 16] The injection of thyroxin into the ovarian capsule alters the morphology of the ovaries in mice.[11, 12]

The present experiment was designed to ascertain the influence of hypo- and hyperthyroid states in young mice upon the effect of a predominantly follicle-stimulating preparation (FSH) on ovarian function.

PROCEDURE

Hybrid female mice (A ♀ X C_3H ♂, AC; and A ♀ X C_{57} ♂, A7, and their reciprocal crosses) were distributed among ten groups when 19 days old. The mice of all groups were sacrificed by illuminating gas when 24 days old. Body and uterine weights were determined at autopsy. Group I (controls) received no treatment. Group II (FSH) received three equal intraperitoneal injections of follicle-stimulating hormone (FSH)‡ dissolved in sterile saline, a total of half an Armour unit, at 12-hour intervals starting at 21 days of age. This dose increased the uterine weight more than 100 per cent.[1] Group III (thyroxin-FSH) received intraperitoneally daily for five days two micrograms of thyroxin¶ dissolved in sterile saline. On the 21st day these mice received FSH as in Group II above. Group IV (thiouracil-FSH) beginning at the age of 19 days, received micro-mixed laboratory chow (Purina) containing three parts thiouracil§

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‡ Armour sterile Pituitary Gonadotrophin, FSH, lyophilized, Lot #377200.

¶ Thyroxin crystals, Squibb, tetraiodo-substituted derivative of Beta- 4-(4'hydroxy phenoxy) Phenyl - alpha-amino-propionic acid.

§ Deracil, thiouracil powder, Lederle.

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per 997 parts of food. When these mice were 21 days old, they received FSH as in Group II above. Group V (thiouracil) received thiouracil as in Group IV but no FSH. Group VI (thyroxin) received thyroxin as in Group III but no FSH. Groups VII-X received two and three times the amount of thyroxin given Groups III and VI (see Table 1).

All animals were kept in an air-conditioned room. The experiments extended over the greater part of a year and animals from the same litter were distributed through several different groups.

TABLE 1. MEAN UTERINE WEIGHT, BODY WEIGHT, AND UTERINE WEIGHT/BODY WEIGHT OF UNTREATED MICE AND OF MICE GIVEN THYROXIN, THIOURACIL, FSH, AND COMBINATIONS OF THE ABOVE

Group and treatment	*Mean body weight (gms.)*	*Mean uterine weight (mgm.)*	*Uterine weight (mgm.) Body weight (gms.)*	*Number of animals*
I—Control	10.6	11.3	1.11 ± 0.20*	103
II—FSH	10.4	30.8	2.96 ± 1.15	120
III—2 μg. Thyroxin FSH	9.7	33.7	3.47 ± 1.36	64
IV—Thiouracil—FSH	9.4	23.3	2.48 ± 0.33	44
V—Thiouracil	9.4	9.2	0.98 ± 0.12	55
VI—2μg. Thyroxin	9.6	13.7	1.43 ± 0.43	61
VII—4 μg. Thyroxin	11.2	11.9	1.06 ± 0.14	69
VIII—4 μg. Thyroxin—FSH	10.7	48.0	4.49 ± 2.17	84
IX—6 μg. Thyroxin	11.6	11.8	1.02 ± 0.14	84
X—6 μg. Thyroxin—FSH	10.1	23.9	2.37 ± 0.61	58

*Standard deviation of the ratios.

Probabilities of significant difference between the uterine weight/body weight ratios of the six groups as determined by t^2 values using the method of Mainland (15) were:

Groups—IV : X, V : IX, VII : IX, not significant
Groups—I : VII, P = 0.10
Groups—II : III, II : IV, P = 0.05
Groups—I : V, I : VI, I : IX, II : VI, II : X, III : VI, III : IX, IV : VI, V : VI, V : VII, VI : VII, VI : IX, VI : X, P = 0.01

All other groups were significantly different at P = 0.01 level.

OBSERVATIONS

The influence of thyroxin on uterine and body weights. The uterine-weight/body-weight (U/B) ratio of immature mice given 2 μg. of thyroxin per day was significantly greater P=0.1) than that of the controls (Table 1). Mice given larger daily doses of thyroxin (4 μg. or 6 μg.) had uteri about the same weight as those of the controls but, because of their greater body weights, had smaller uterine-weight/body-weight ratios. Although mice of the latter two groups (VII and IX, Table 1) were

heavier than those receiving the smallest dose of thyroxin, their uteri weighed about 2 mgm. less than those of the mice given 2 μg. of thyroxin.

Influence of thiouracil on uterine and body weight. The U/B ratios of mice fed thiouracil and the absolute body weight and uterine weight were lower than those of the controls (P=0.01). The mean uterine weight of the hypothyroid mice (Group V, Table 1) was 4.5 mgm. smaller than that of the mice given 2 μg. per day of thyroxin (Group VI). The ratios were quite constant among the groups not given FSH, the standard deviations of the ratios ranging from 0.12 to 0.20.

Influence of FSH at different levels of thyroid function. The U/B ratios of the groups given FSH (II, III, VIII, and X, Table 1) were always increased over that of the respective controls (I, V, VI, VII, and IX, Table 1). The variation of the response within the FSH-treated groups was greater than in the groups with only their intrinsic gonadotrophins (Table 1). The uteri of the mice given 2 μg. thyroxin in addition to FSH weighed about 3 mgm. more than those receiving FSH alone, and the U/B ratios were significantly different (P=0.05). Larger doses of thyroxin (4 μg. daily) resulted in more variable but larger uterine responses in the mice given FSH. The difference in U/B ratio was highly significant (P=0.01), and the mean uterine weight of animals given 4 μg. thyroxin and FSH was over 17 mgm. greater than the mean uterine weight of mice given FSH alone. Mice given 6 μg. of thyroxin had a smaller uterine response to FSH than did the mice given FSH alone or FSH in conjunction with the two smaller doses of thyroxin.

Influence of thyroid levels on body weight. The mean final body weight of the untreated control mice was 10.6 gms. The mean final body weights of all groups of treated animals ranged within ± 1.2 gms. from the controls. Consistent differences between the body weights of thyroxin-treated mice were lacking when all dosages and thyroxin-FSH groups were considered (Table 1). Animals given 4 μg. and 6 μg. thyroxin daily showed variable increases in body weight over controls. Thiouracil-treated mice consistently weighed less than the controls.

Possibility of strain differences in responses. The U/B ratios were higher in A_7 mice than in AC mice (A_7 controls—1.24; AC controls—1.13; A_7 FSH-treated—3.27; AC FSH-treated—2.90) but all values are within a standard deviation from the composite means. The mean U/B ratios of the ten animals with the smallest body weight and the ten animals with the largest body weight were within one standard deviation of the group mean (controls—smallest 1.17; largest 1.12; FSH-treated—smallest

3.59; largest 2.72). FSH treatment exaggerated the strain variation and increased the range of variation within the strain.

Differences in body weight were equalized by the use of the U/B ratio, but the dosage of thyroxin was calculated for a hypothetical 10.0 gm. mouse and not equilibrated to individual variation in body weight. Two μg. per day of thyroxin is more than twice the estimated output of d,l-thyroxin (9.2 micrograms/100 gms. body weight) for five-week-old mice.[14]

Influences on ovaries. Corpora lutea and blood points were noted at autopsy, most frequently among the mice given FSH and 4 μg. of thyroxin per day (more than 40 per cent) and less frequently among the mice given FSH and 2 μg. thyroxin daily (more than 30 per cent). Neither corpora lutea nor blood points appeared among the mice given FSH and 6 μg. of thyroxin. Corpora lutea were observed occasionally (less than 25 per cent) among mice given FSH alone and very rarely among mice of other groups.

DISCUSSION

The effect of FSH on ovarian function, as measured by changes in the uterine-weight/body-weight (U/B) ratio of immature female mice, is influenced by thyroxin and thiouracil treatment. The U/B ratio of immature female mice, is influenced by thyroxin and thiouracil treatment. The U/B ratios of mice receiving a given dosage of FSH were increased by administering 2 or 4 μg. thyroxin daily and decreased by administering 6 μg. thyroxin daily or by thiouracil treatment. Mice given the smallest doses of thyroxin showed evidence of an augmented uterine response to gonadal hormones. Either optimal levels of thyroid function were not attained for maximal response to intrinsic gonadotrophic hormones, or added thyroxin augmented the production of additional intrinsic gonadotrophin, or modified the responsiveness of the ovaries or uteri. The effect of thyroxin treatment is dependent on the dosage. The larger doses of thyroxin (4 μg. and 6 μg.) did not show a similar effect. It has been shown that mice may not have adequate thyroid levels for the usual responses to larger doses (10 I.U.) of PMS.[8] When thyroprotein was fed in adequate amounts, the ovarian response to constant amounts of gonadotrophin was increased.[8]

FSH presumably acts directly on the ovary to increase the production of estrogen which in turn stimulates uterine growth (weight). The exogenous thyroxin may act on any or all of the following: pituitary, ovaries, or uterus. Thyroxin could act directly on the pituitary (a) by stimulating increased gonadotrophin (FSH or LH) production, or (b) by disturbing a balance between thyrotrophic hormone production and gonadotrophin

production, it being assumed that the injection of thyroxin would decrease thyrotropic hormone production and thereby might permit an increased gonadotrophin production. The thyroxin could (a) potentiate the action of gonadotrophin on the ovaries to increase estrogen production, or (b) directly stimulate the ovaries to produce estrogen. The thyroxin could (a) potentiate the action of estrogen on the uterus with resultant increased growth (weight), or (b) directly stimulate uterine growth (weight). The increased frequency of the formation of corpora lutea and of appearance of blood points among the thyroxin-FSH group indicates that thyroxin could act by increasing the LH secretion of the pituitary as has been suggested by other experiments as well as those reported here. Others have noted the induction of typical pseudo-pregnancy with large functional corpora lutea in albino rats by means of experimental hyperthyroidism.[11] On the other hand, the inhibition of the ovarian response of rats in parabiosis with intact or castrate females by the injection of either partner indicated that added thyroid acts upon the ovarian response to gonadotrophin.[10]

The observations reported here were made on young animals in rather brief experiments. The time factor has differed in many of the experiments that have been reported in the past and animals have been maintained at different levels of exposure to thyroid hormones for variable periods of time prior to exposure to gonadotrophic or gonadal hormones. This may account for some of the contrasting observations that have been reported.

The small but significant increase in uterine weight, when small amounts of thyroxin are given, but the lack of any effect when larger amounts were given, indicated that thyroid has little effect in young female mice of activating the pituitary gland. On the other hand, thyroxin seemed to augment the effect of injected FSH on the ovaries as observed histologically and also as determined secondarily by the increased uterine weights. One might assume that thyroxin increases ovarian responsiveness to FSH. The possibility does remain that this may be the result of the production of a small amount of LH by the animal's own pituitary gland which augments the response of the injected FSH.

SUMMARY

1. Hybrid immature female mice given daily intraperitoneal injections of 2 or 4γ of thyroxin showed a greater but more variable uterine response to FSH than did animals given FSH alone. Six μg. of thyroxin administered daily reduced the uterine response of mice given FSH. The effect of daily thyroxin treatment on the mice with or without exogenous FSH is therefore dependent on the dosage administered.

2. Thiouracil decreased the uterine response to injected FSH.

3. Among mice not given FSH, thiouracil reduced the uterine weight/body-weight ratio and 2γ thyroxin daily augmented it, whereas 4 or 6γ thyroxin reduced it.

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THE AUDITORY MANIFESTATIONS OF LEUKEMIA

The otological complications of leukemia are common, but rarely are they given much consideration in discussions of the clinical aspects of leukemia. During the past several years the frequency with which deafness, tinnitus, and vertigo have developed in many of our patients has prompted us to investigate the incidence and type of involvement which occurs in this disorder.

The aural manifestations of leukemia are of considerable historical interest. The first case of leukemia ever described was that of a 44-year-old female who was admitted to a hospital because of an abdominal tumor and deafness of 6 months' duration in the year 1844.[9] The tumor proved to be an enlarged spleen, and blood studies at autopsy by Donné showed an excessive leukocytosis. Only twelve years later Vidal[36] reported deafness in a case of leukemia and was able to find 3 more in a total of 32 cases of leukemia. In 1906 Alexander[3] pointed out that the probable etiology of the first case of Ménière's syndrome was acute leukemia. The case was that of a young girl who developed sudden deafness, severe vertigo, and vomiting, which was followed by death five days later. In Ménière's first report on the autopsy findings[30] he stated that a red plastic material filled the entire labyrinth, but in a later report[37] he said that the red substance was found only in the semi-circular canals without demonstrable abnormalities of the vestibule and cochlea. Alexander believed that the red plastic material was blood which had remained following sudden hemorrhage into the labyrinth as a complication of leukemia. He felt that the Ménière's syndrome must have developed as a terminal complication of a fatal process, since death could not have resulted from the syndrome per se. More recently, Alexander's interpretation of Ménière's case has been challenged.[30] Much of the early medical literature on leukemia described the aural symptoms and pathological changes which developed as complications of leukemia. Between the time of Vidal's report and the turn of the cen-

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tury a number of individual case reports, small series reports both with and without pathological correlation, have appeared in the literature.[3, 7, 21, 22, 24, 27, 31, 46, 50, 52, 57] These articles on the subject described the various clinical and pathological patterns but have contributed little in terms of therapy. During the past fifty years many other interesting reports [1, 4, 5, 6, 8, 10, 11, 12, 14, 20, 23, 25, 26, 28, 29, 32, 35, 38, 45, 47, 49, 51, 53, 55, 58] have appeared on this subject. Forkner[15] in 1938 made a review of much of the older literature

TABLE 1. AUDITORY DISORDERS AND LEUKEMIA

		Patients with auditory disorders		
Type of leukemia	*Total no. cases*	*Total no.**	*No. developing before diagnosis of leukemia*	*No. developing after diagnosis of leukemia*
Chronic lymphocytic	52	15	2	11
Subacute lymphocytic	5	..	..	..
Acute lymphocytic	9	3	1	2
Chronic monocytic	1	1	..	..
Acute monocytic	8	2	..	1
Chronic granulocytic	10	5	1	3
Acute granulocytic	8	6	2	4
Unclassified	7	..	..	..
Totals	100	32	6	21

* Auditory symptoms in 5 of these patients appeared unrelated to the development of leukemia.

and in 1945 Druss[10] found the otological complications of leukemia to have an incidence of 16.5 per cent in a random group of 148 patients.

The primary objective of this study is to emphasize the importance of certain acoustic disturbances as either primary or secondary manifestations of leukemia. It is hoped that definitive therapy will be facilitated through the proper recognition and understanding of these difficulties.

METHOD OF STUDY

The records of the last 100 patients with leukemia admitted to the Yale-New Haven Medical Center were reviewed carefully for evidence of any acoustic difficulties. All patients with leukemia, irrespective of age, sex, and type of leukemia, were included. The various types of leukemia represented and the relationship between the date of diagnosis and the appearance of otological complications are shown in Table 1.

RESULTS

Thirty-two of the patients in this group had definite auditory signs or symptoms. In 16 there was a well-established relationship between the leukemia and the development of these disorders (Table 2). In an additional 11 there was an almost certain but less definite relationship (Table 3). There were 6 instances in which an otological complication presented as the only symptom or as part of the initial complaints which brought the patient to the hospital. Four of the 6 had acute leukemia. The remaining developed their auditory difficulties sometime during the course of their illnesses. In 5 of the 32 patients either the characteristics of the disorder were poorly documented or it was reasonably clear that the leukemia was not responsible.

The most frequently encountered symptoms in the 27 patients with auditory complications were deafness in 16, tinnitus in 7, and pain in 3. Only one patient in the entire group complained of vertigo. A few patients were asymptomatic but had bleeding or purulent drainage from an external canal. The term deafness is employed to imply varying degrees of hearing loss.

DISCUSSION

The incidence of otological complications due to leukemia in this small series is 27 per cent. As unusual feature of this series was the infrequent occurrence of vertigo. In a series of 39 patients Love[35] found vertigo in 28, but it is likely that many of his cases were not due to leukemic involvement of the ear. In his paper he mentions that slight dizziness without associated auditory symptoms is common in leukemia, and that probably it is due to cerebral anemia and general debility. In spite of the fact that our case histories usually were detailed and complete, it is possible that the auditory troubles of additional patients may not have been recorded. Without the aid of histological examination it was difficult to be sure of the pathophysiology of some of the instances in which acoustic difficulties were encountered. In general, however, it is clear that patients in whom hemorrhage was responsible usually had acute leukemia. On the other hand, obstruction of the Eustachian canals due to lymphoid hyperplasia was encountered only in chronic lymphocytic leukemia. Infection of the middle ear showed no predisposition to any particular type of leukemia. An evaluation of these complications indicates that auditory difficulties are more common and develop earlier in patients with acute leukemia than in those with chronic leukemia.

TABLE 2. CHARACTERISTICS OF AUDITORY DISORDERS DEFINITELY RELATED TO LEUKEMIA

Patient	*Age*	*Sex*	*Type of leukemia*	*Symptoms*	*Cause*	*Remarks*
J.O.	26	M	Chronic granulocytic	Deafness	8th nerve hemorrhage	Developed rapidly as initial symptom of leukemia.
W.M.	33	M	Chronic granulocytic	Deafness	Eustachian tube obstruction	Bilateral, severe. Onset with development of skin lesions.
F.F.	59	M	Chronic lymphocytic	Deafness	Lymphoid hyperplasia	Initial symptom of leukemia. Cleared with x-ray to tonsils.
J.D.	58	M	Chronic lymphocytic	Tinnitus	Lymphoid hyperplasia	Cleared after x-ray to tonsilar areas.
A.Z.	60	M	Chronic lymphocytic	Purulent drainage	Otitis media	Drainage cleared with antibiotic therapy.
A.A.	76	M	Chronic lymphocytic	Deafness	Otitis media, infiltration	Rapid unilateral loss with BC > AC. No improvement.
F.Fr.	69	M	Chronic lymphocytic	Tinnitus, deafness	Lymphoid hyperplasia	Tinnitus gradually cleared, but bilateral deafness became worse.
P.B.	65	M	Chronic lymphocytic	Deafness	Lymphoid hyperplasia	Had paralysis of 9th and 10th nerves on left due to lymphoid hyperplasia in neck and nasopharynx.
M.T.	54	F	Chronic lymphocytic	Deafness and pain	Lymphoid hyperplasia and infection	Bilateral perceptive hearing loss. Conductive loss, right.
K.T.	2½	F	Acute granulocytic	None	Middle ear hemorrhage	Blood behind both membranes. Improved with Rx.
B.B.	23	F	Acute granulocytic	Tinnitus, deafness	Otitis media	Initial good response, but terminal severe deafness.
W.C.	42	M	Acute granulocytic	Tinnitus, pain	? Hemorrhage	Sudden onset. Moderately severe and persistent.
E.M.	36	F	Acute granulocytic	External bleeding	Hemorrhage	Continuous bleeding from right external auditory canal.
J.S.	3½	M	Acute lymphocytic	Pain	Serous, otitis media	Sudden onset as initial symptom of leukemia.
K.S.	34	F	Acute lymphocytic	Deafness	Hemorrhage	Terminal bleeding into left middle ear.
P.S.	2½	F	Acute lymphocytic	None	Otitis media	Intermittent injection of drums with fluid on left.

TABLE 3. CHARACTERISTICS OF AUDITORY DISORDERS PROBABLY RELATED TO LEUKEMIA

Patient	*Age*	*Sex*	*Type of leukemia*	*Symptoms*	*Probable cause*	*Remarks*
J.D.	63	M	Chronic granulocytic	Tinnitus, deafness	Leukemic infiltration	Unilateral deafness. High-pitched continuous tinnitus.
S.G.	52	M	Chronic granulocytic	Tinnitus	?	Sudden onset of persistent "banging in ears."
J.K.	53	F	Chronic lymphocytic	Deafness	?	Recent gradual onset of moderate unilateral deafness.
J.R.	72	M	Chronic lymphocytic	Deafness	?	Gradual onset of bilateral perceptive hearing loss.
I.M.	57	M	Chronic lymphocytic	Deafness, vertigo	?	Gradual loss on left and sudden on right.
F.C.	38	F	Chronic lymphocytic	Deafness	Infiltration, Otitis media	Moderately rapid loss, BC > AC.
M.L.	66	F	Chronic lymphocytic	Deafness	Otitis media	Bilateral repeated infections for 2 years prior to diagnosis.
R.D.	61	F	Chronic lymphocytic	Deafness	?	Bilateral air conduction loss with onset prior to diagnosis.
W.D.	19	M	Acute granulocytic	None	Otitis media and mastoiditis	Both conditions diagnosed at onset of leukemia.
C.K.	5	F	Acute granulocytic	Deafness	?	Bilateral loss antedating dx. of leukemia by few months.
H.H.	57	F	Acute monocytic	Tinnitus	Anemia	"Roaring and rushing" sounds for 1 week prior to death.

The mechanisms by which the auditory system may become involved are varied. Although the cause of the otological complication could not be established in many of our cases, it appears that the underlying pathological mechanisms fall into one or a combination of three main groups, neoplastic invasion, infection, and hemorrhage.

LEUKEMIC INVASION

Diffuse or focal leukemic invasion of the external ear,[10, 35] middle ear,[10, 14, 35, 55] or internal ear[4, 10 25, 28, 31, 35, 48, 50, 57] structures as well as the 8th nerve[3, 8, 18, 28, 39, 48] may occur. Invasion of the soft tissues, membranes, hollow spaces, and marrow of ossicles may develop. These infiltrates may be

asymptomatic or may result in deafness, tinnitus, pain, or vertigo. Although post-mortem examination of the auditory system was not done in our cases, it is probable that leukemic infiltration of the cochlea was the cause of deafness and tinnitus in many of them.

A few cases of chronic granulocytic leukemia have been recorded in which chloromas have produced auditory changes.[6, 17, 19, 28, 33, 38, 40, 44, 45] The most frequent location for these tumors has been in the orbit, mastoid, or internal auditory meatus. These have produced real or apparent mastoid and middle ear infections, pain, deafness, vertigo, and pressure symptoms on the 7th and 8th nerves. The presence of tumor nodules in the ear has been recorded in a case of chronic lymphocytic leukemia.[55] Pearson[44] has pointed out that about 50 per cent of patients with chloromas will have involvement of the temporal bone. He also has emphasized the predelection of this disorder for young males.

Three patients in this series developed deafness secondary to Eustachian tube obstruction produced by either enlarged leukemic tonsils or from leukemic nodules in the nasopharynx. Blockage of an Eustachian tube usually is followed either by a serous effusion or active bacterial infection in the middle ear. It is probable that some of the cases of otitis media reported in leukemia have been the result of a similar mechanism. On the other hand, extreme involvement of the tonsils may develop without apparent effect on any of the auditory mechanisms.[54] The two patients with tonsillar enlargement had chronic lymphocytic leukemia, whereas the patient with nasopharyngeal nodules had terminal chronic granulocytic leukemia. Examples of this syndrome are illustrated by the following case reports.

CASE #1

F.F. was a 59-year-old white male who was first seen in the ENT clinic of Yale-New Haven Medical Center because of impaired hearing over a period of five months. The deafness had come on gradually but had progressed in severity and was associated with anosmia, hoarseness, and dysphagia. There was a history of a 19-pound weight loss in six months. Five weeks prior to the clinic visit he was treated with penicillin for tonsillitis by a physician with improvement in his symptoms. Examination by an otolaryngologist at the clinic revealed multiple enlarged lymph nodes in the anterior and posterior cervical triangles. The tonsils were very enlarged bilaterally. The nasopharynx was partially filled with masses of lymphoid tissue which occluded the Eustachian tube orifices. Both tympanic membranes appeared dull. Bone conduction was greater than air conduction. A biopsy of the nasopharyngeal lymphoid tissue suggested lymphocytic leukemia. The total leukocyte count was 35,200/mm^3 with 90 per cent lymphocytes. The patient was begun on x-ray therapy to the neck and within two weeks showed considerable improvement as the lymphoid tissue regressed. His hearing

returned to normal and the dysphagia, anosmia, and hoarseness disappeared. The white blood count dropped to 6,400/mm³ one month later with a decrease in the lymphocyte count to 52 per cent. He remained well for six months, then began to complain of malaise, anorexia, and pain in the abdomen, probably the result of splenic infarcts. Four years later he had a recurrence of moderate deafness associated with hoarseness and dysphagia. Physical examination revealed diffuse hyperplasia of the nasopharyngeal lymphoid tissue; both Eustachian tubes were blocked. He again was given irradiation to the neck with considerable improvement in the obstructive symptoms. His disease, however, continued to progress at a rather rapid rate; he became cachectic and expired four years after original diagnosis had been made.

COMMENT

This patient had a conductive type of deafness resulting from Eustachian tube obstruction due to enlarged nasopharyngeal tissue. The appearance of the tympanic membranes indicated the presence of a serous effusion in the middle ear. Biopsy of the nasopharynx showed lymphatic leukemia. X-ray therapy to the nasopharyngeal areas on two separate occasions resulted in improved hearing.

CASE #2

W.M., a 36-year-old white male, was admitted to the Yale-New Haven Medical Center in the terminal stage of granulocytic leukemia. He was diagnosed as having chronic granulocytic leukemia in October 1951, and had been treated successfully during the next four years with splenic irradiation and Myleran. In the course of his final hospitalization he developed an acute blastic crisis and a striking nodular leukemia cutis over his entire body. Over a period of several days the patient developed gradually increasing deafness. Examination of the pharynx revealed many yellow-white nodules, about 1 cm. in diameter, situated on the palate and nasopharynx, some of which were occluding the Eustachian tubes. He was started on a course of demecolcin ("Colcemide" —Ciba). There was a striking regression of the skin and mucous membrane lesions and a concomitant moderate improvement in his hearing. In spite of his brief improvement he continued to run a downhill course and expired.

COMMENT

This was an unusual case since nodular leukemic infiltrates are uncommon in chronic granulocytic leukemia. These lesions were found in the nasopharynx and also strategically located about the orifices of the Eustachian tubes. It is quite possible that some of the infiltrates were located within the tubes themselves. Following therapy with demecolcin, the nodules regressed with a subsequent moderate improvement in hearing. It should be pointed out that the patient also exhibited widespread hemorrhagic manifestations and it is probable that hemorrhage in or about the acoustic structures may have contributed to his deafness.

INFECTION

Bacterial infection involving the middle ear[10, 12, 14, 16, 28, 38, 44, 49] and mastoid[1, 10, 12, 38] is quite common in acute leukemia. In many instances it is secondary to infiltrative obstruction of vascular channels and certain orifices, to hemorrhages, or to greatly reduced systemic resistance to infection. Inflammatory lesions of the middle ear tend to invade rapidly,[10] particularly into the mastoid spaces. Mastoiditis may be extremely difficult to diagnose, since mastoid chloromas, post-auricular swelling, and tissue sagging occasionally develop in leukemia.[10, 12, 17, 19, 38, 45] A recent publication reported the case of an 11-year-old boy who developed severe otitis media, mastoiditis, and lateral sinus thrombosis before the diagnosis of acute leukemia was made at autopsy.[12] Several of our cases developed deafness as a result of middle ear infections and some of these responded to antibiotic therapy. Many of these cases developed mild upper respiratory infections which resulted in otitis media. In some, this was a presenting complaint and in others it became the most troublesome symptom. There did not appear to be any obvious correlation with the phase of the disease or type of leukemia.

CASE #3

B.B. was a 24-year-old white female diagnosed as having acute granulocytic leukemia in July 1954. She had been treated with cortisone and 6-mercaptopurine with fair success until November 1955, at which time she began to complain of deafness. Examination at this time showed moderate hypertrophy of the pharyngeal tonsils and some reddening of the eardrums. She gradually developed pain in both ears followed by a purulent discharge. In spite of intensive antibiotic therapy there was only temporary relief of the bilateral otitis media. Within the next few months she had repeated ear infections with spontaneous perforation of her tympanic membranes and the hearing loss became more severe. There was considerable sanguino-purulent discharge from both ears. Culture of this material showed mixed organisms. The ear infections continued and she developed a left mastoiditis which drained spontaneously to the skin. She continued a downhill course and expired in April 1956.

COMMENT

The otitis media and mastoiditis proved to be the most troublesome manifestations of the late stage of leukemia in this patient. The prolonged administration of cortisone and antibiotics may have enhanced her susceptibility to ear infections with resistant organisms. In addition her leukocyte phagocytic mechanisms were severely impaired throughout the entire course of her illness. It is very probable that this type of localized infection with mixed resistant organisms will be encountered more frequently

in the future with the increased use of cortisone and various antibiotic and chemotherapeutic agents.

HEMORRHAGE

Hemorrhage may occur into practically all structures of the ear. It probably develops much more frequently than is clinically evident, since bleeding into many areas may be completely asymptomatic. A number of cases, however, in which vertigo, deafness, and tinnitus have developed following intraural hemorrhage have been reported.[2, 7, 10, 13, 15, 22, 29, 34, 35, 41, 43, 46, 53] Labyrinthine hemorrhage probably is a relatively rare complication of leukemia. Fraser[16] observed only two cases in the course of examination of 35,000 patients who were studied for affections of the ear.

Impaired function may result from multiple small hemorrhages, a massive single hemorrhage, or from eventual fibrosis and calcification of these hemorrhages (osteitis interna ossificans).[2, 13, 20, 22, 26, 28, 35, 42, 43, 46, 53] Not infrequently bleeding may precede or accompany infection. Sudden onset of acoustic disturbances in association with a generalized hemorrhagic diathesis always should suggest the possibility of middle or inner ear bleeding due to leukemia.

CASE #4

A 26-year-old white male (J.O.) was first seen at the New Haven Hospital ENT Clinic in September 1953 because of sinus trouble and deafness. Five weeks earlier he had noted the sensation of "hollowness" in his right ear. The next morning he awakened deaf. Five days later he had a sudden decrease in vision, especially on the right. His past history was significant in that a chronic mastoid infection during childhood had left him completely deaf on the left. On physical examination there was some thickening of the right ear drum. Air conduction was diminished but remained greater than bone conduction. Fundoscopic examination showed numerous bilateral hemorrhages and exudates. A firm spleen was palpated 5 cm. below the left costal margin. Routine hematology studies showed a hemoglobin of 9.0 gms. per cent, total leukocyte count of 660,000/mm^3 with a distinct left shift. He was admitted to the medical service for further study and management. Ophthalmological findings were considered to be consistent with "leukemic retinopathy." Sternal marrow examination confirmed the diagnosis of chronic granulocytic leukemia. Following blood transfusions and splenic irradiation there was improvement in the deafness to the extent that he was able to hear loud voices. An audiogram showed that he was able to respond to 60 decibels on the right. Subsequent to the completion of x-ray therapy to the spleen he was given a course of radioactive phosphorous with excellent hematologic remission. Three months later there was a return of clinical and hematologic manifestations of leukemia and he was begun on a course of Myleran with good response. Maintenance Myleran therapy controlled the leukemia during the ensuing three years with no change in his hearing problem. In March 1957 he developed an acute relapse, deteriorated rapidly, and expired following a cerebral vascular accident.

COMMENT

The deafness in this case was probably due to a combination of hemorrhage and leukemic infiltration. The fact that there was some improvement in hearing following therapy suggests that a conductive defect due to neoplastic invasion may have been present. In favor of a hemorrhagic etiology was the sudden onset of deafness, evidence of hemorrhagic tendencies, as manifested by the retinopathy and continued oozing from the sternal marrow aspiration site, and permanency of the hearing loss. On the other hand, the sudden onset of deafness, per se, does not necessarily imply hemorrhage as has been illustrated recently by de Stefani.[8] He described the sudden onset of bilateral deafness in a 33-year-old male, due entirely to leukemic infiltration in the cochlear-vestibular regions with serous inflammation of the acoustic nerves.

THERAPEUTIC CONSIDERATIONS

Usually it is difficult to decide on the appropriate specific form of therapy for the acoustic complications of leukemia. Obviously the proper therapy depends on accurate diagnosis, and often the etiology of the lesion is obscure. The abrupt, painless onset of symptoms in association with a hemorrhagic diathesis usually points to hemorrhage. Attention should be directed toward correcting a specific clotting factor deficiency, neutralizing a circulation anticoagulant, and treatment of the leukemia. Frequently the administration of prednisone and prednisolone will reduce capillary bleeding and may be of great value in preventing additional bleeding into ear structures. Little can be done for the more chronic acoustic disturbances which accompany calcification and fibrosis of the middle or inner ear structures. If these changes are secondary to repeated hemorrhages, every attempt should be made to modify the clotting defect in order to prevent their progression.

Middle and external ear infections develop more insidiously, frequently perforating the tympanic membrane and invading adjacent structures rapidly. Severe pain, high fever, and conductive type hearing loss are not uncommon. The principal treatment of these infections is the use of appropriate antibiotics. The bacterial organisms isolated, however, usually are considered to be of low pathogenicity and may be extremely resistant to antibiotic therapy.

Acoustic complications due to leukemic infiltration of tonsils, adenoids, external, middle, or inner ear structures are considerably more amenable to therapy than are those due to hemorrhage. Frequently systemic leukemia therapy will cause regression of the infiltrated areas and improve-

ment of the auditory difficulties. Sometimes, however, it becomes necessary to direct irradiation therapy to the internal ear or to irradiate enlarged adenoid and tonsillar tissue locally. Rapid relief of Eustachian tube obstruction may prevent the development of severe otitis media. If middle ear infection is associated with this type of obstruction, antibiotics will rarely be completely effective unless the obstruction is relieved.

Perceptive deafness due to hemorrhage or leukemic infiltration of the 8th nerve or its center usually is a late complication of leukemia. Except for systemic control of the leukemia and the hemorrhagic diathesis, little can be done to reverse this situation.

SUMMARY

1. Twenty-seven of 100 consecutive patients with leukemia were found to have acoustic disturbances.

2. Two cases of hearing loss in chronic lymphocytic leukemia secondary to leukemic infiltration of tonsillar and adenoid tissue have been described.

3. The most frequently encountered aural manifestations of leukemia were hearing loss, tinnitus, and pain. Vertigo developed in only one patient.

4. Auditory complications developed earlier and more frequently in the acute than in the chronic leukemias.

5. Auricular or periauricular structural derangement due to hemorrhage, neoplastic invasion, and/or infection appears responsible for the auditory manifestations of leukemia.

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HELLMAN COLLECTION OF EARLY OBSTETRICAL BOOKS

In the December 1951 issue of *The Yale Journal of Biology and Medicine* (*24*, 210-219) the late Dr. Alfred M. Hellman of New York described his important collection of early obstetrical books. This formed an introduction to a bibliography of his collection which Dr. Hellman distributed to any who were interested in his books. The recipients of his publication will be glad to hear that his daughter, C. Doris Hellman, has written an addendum to her father's work which will appear in the *Academy Bookman*, 1958, volume 11, number 2, under the title "Additions to the Alfred M. Hellman Collection of Obstetrical Books." Reprints will be available and can be obtained from C. Doris Hellman, c/o Mrs. Morton Pepper, 1065 Lexington Avenue, New York 21, New York.

It is pleasant to know that Dr. Hellman's collection will remain intact in the library of the London obstetrician and gynecologist, Mr. Alistair Gunn.

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COMPATIBILITY FACTORS INFLUENCING THE ACCEPTANCE OF RAT BONE MARROW GRAFT BY THE IRRADIATED MOUSE**

Heavily irradiated mice have been protected from otherwise lethal bone marrow damage by intravenous injection of rat bone marrow.[1, 4, 5, 8, 13, 14] It has been amply demonstrated that the radio-protection offered by the rat bone marrow is actually due to myelopoietic repopulation of the mouse marrow cavity by rat marrow precursor cells.[1, 4, 5, 8, 13, 14] Mice given supralethal irradiation plus rat bone marrow have been demonstrated to produce rat erythrocytes,[4, 5, 8, 14] rat granulocytes,[7, 12] and rat platelets.[9]

This rather bland acceptance of rat bone marrow by the irradiated mouse naturally led to experiments designed to test the possibility that bone marrow from other heterologous species would "take" and grow in the irradiated mouse. In our laboratory we have found that marrow from humans, guinea pigs, rabbits, and hamsters all failed to prevent lethal marrow damage in the irradiated mouse, and these same negative results have been reported by others.[12]

It seemed quite evident, then, that rat bone marrow is the only "heterologous" marrow that has been found acceptable to the irradiated mouse. The studies reported in this communication deal with the compatibility factors between rat and mouse that allow this inter-species marrow graft acceptance.

MATERIALS AND METHODS

All mice were inbred strains (C57 Black X 101 or C3H X 101 hybrids) obtained from Cumberland View Farms, Clinton, Tenn. Both male and female mice 10-14 weeks of age were used in various experiments.

As many as 15 mice were irradiated together in a plastic box. Irradiation was carried out using a 200 kilovolt standard therapy machine (15 milliamps; 0.5 mm. copper and 1.0 mm. aluminum filter; 50 cm. tube distance).

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Total dosage was calculated as 700 roentgens, a dose at least 200 roentgens beyond the 100 per cent lethal point in our system of irradiation. In biological effect, our 700 roentgens compared with 900-950 roentgens used by Congdon[9] in mice of the same strain from the same supplier, in that mortality in untreated mice occurred 7-10 days' post-irradiation. Doses of 900-950 roentgens as measured output by our instruments produced early death, and poor protection was achieved by marrow infusion from donors of the same strain (isologous marrow treatment). The cause of this discrepancy in irradiation dosimetry has not been determined, but van Bekkum[12] found similar dosages necessary in his experiments.

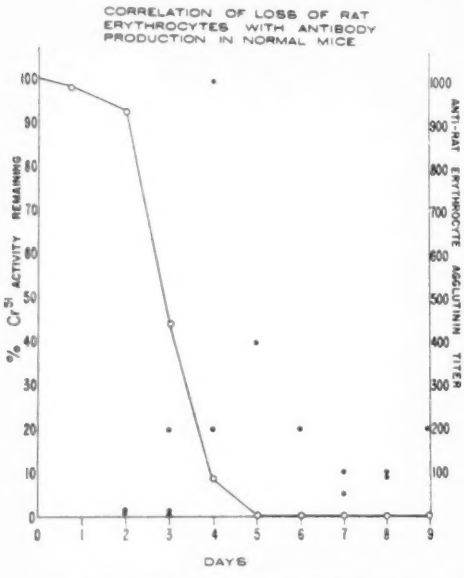

FIG. 1. Correlation of loss of rat erythrocytes with antibody production in normal mice.

Bone marrow suspensions from mice were prepared according to the method of Congdon.[9] Femoral marrow was expressed from the bone shaft after the bone was cut at the upper end. A size 23 needle attached to a tuberculin syringe containing Hank's solution was inserted through the tibial end of the bone, and the marrow expressed into a small beaker. The marrow plugs were separated by drawing the solution in and out of the syringe repeatedly. Final values were adjusted so that one femoral marrow was contained in one-half milliliter of Hank's solution. This dosage, containing approximately 5-7 million nucleated marrow cells, was the usual mouse marrow inoculum used.

In studies involving the destruction of red blood cells the erythrocytes to be tested were labeled with radioactive sodium chromate (Abbott) and injected intravenously into the recipient mice. Injection of test cells in irradiated mice followed the irradiation by only a few minutes. Donor bloods, taken with heparin as an anticoagulant, were incubated for one-half to one hour with $Na_2Cr^{51}O_4$ in sufficient quantity to insure that each recipient mouse would receive 0.5-1 microcurie of radioactive material in 0.5 cc. of washed labeled donor cells having a 30-50 per cent hematocrit. Determinations of blood radioactivity in the recipients were made by collecting the blood after decapitation and counting the sample thus obtained in a well-type scintillation counter. Counts from the blood of 2-6 individual mice were averaged and expressed as per cent of dose injected, assuming a total blood volume of 2.0 ml.

Specific applications of these methods, as well as certain other techniques used, will be elucidated in the text.

RESULTS

A. Rodent erythrocytes in the mouse

Labeled rat erythrocytes given to normal mice persisted in the circulation for about three days and then rapidly and completely disappeared. Mouse blood volumes calculated from the labeled rat erythrocytes 24 hours after injection were approximately of expected theoretical values, indicating no sizeable immediate loss of rat erythrocytes from the circulation. The rapid disappearance of rat erythrocytes after the third day was associated with the appearance of anti-rat erythrocyte agglutinins of variable titre in normal mice (Fig. 1).

TABLE 1. LIFESPAN OF ERYTHROCYTES PER CENT Cr^{51} ACTIVITY REMAINING

Days' post infusion	*Rat (autologous)*	*Mouse (isologous)*	*Mouse (homologous)*	*Rat cells in irradiated mouse*
1	80	85	100	105
2	96	58	...	117
3	78	..	...	88
4	..	..	89	79
5	..	..	...	94
6	..	..	86	97
7	..	66	...	66
8	..	..	62	64
9	69	90	...	...
11	..	73	...	...
12	..	..	74	...
13	51	..	...	...
14	..	43	...	30
15	..	..	...	29
16	45	55	66	...
18	..	58	...	27
20	37	..	39	...
21	..	42	...	...
23	34	37	19	...
25	..	..	...	15

When labeled rat erythrocytes were injected into irradiated, isologous-marrow-treated mice, the lifespan of cells was not markedly decreased, and no demonstrable anti-rat erythrocyte agglutinins appeared in the serum. Indeed, the disappearance rate of labeled rat erythrocytes in the irradiated mouse was similar to the curve of disappearance of chromium-51 tagged isologous and homologous mouse erythrocytes and autogenous rat erythrocytes (Table 1).

Erythrocytes from other species were tagged and similarly injected into normal mice. Human, guinea pig, rabbit, and chipmunk erythrocytes were lysed almost immediately by the normal mouse (Fig. 2). Although some hamster erythrocytes persisted in the circulation for several days, there was a large initial loss of cells. Irradiated mice did not accept the foreign erythrocytes any better than the normal mice, except that there was possibly some prolongation of hamster erythrocyte lifespan in irradiated mice (Fig. 3).

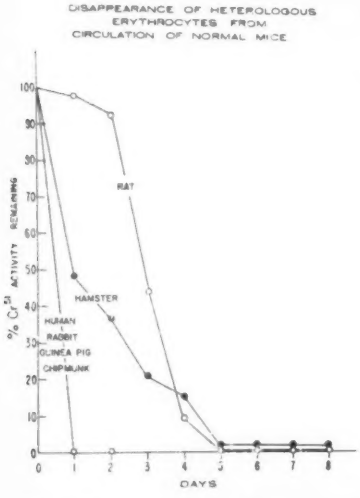

FIG. 2. Disappearance of heterologous erythrocytes from circulation of normal mice.

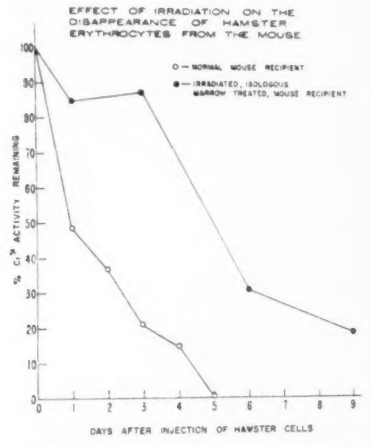
EFFECT OF IRRADIATION ON THE DISAPPEARANCE OF HAMSTER ERYTHROCYTES FROM THE MOUSE
O — NORMAL MOUSE RECIPIENT
● — IRRADIATED, ISOLOGOUS MARROW TREATED, MOUSE RECIPIENT
% Cr51 ACTIVITY REMAINING
DAYS AFTER INJECTION OF HAMSTER CELLS

FIG. 3. Effect of irradiation on the disappearance of hamster erythrocytes from the mouse.

Correlation of these *in vivo* results with *in vitro* naturally occurring agglutinins or hemolysins was not successful. Fresh frozen normal mouse serum (0.2 cc.) incubated for 24 hours with 0.1 cc. of 5 per cent heterologous erythrocytes suspended in saline, caused neither agglutination nor hemolysis of human, guinea pig, rat, or homologous mouse cells, but did cause agglutination of hamster erythrocytes.

B. Studies of heterologous antisera

Antisera were produced in rabbits to a number of different rodent erythrocytes or rodent sera. These antisera were then tested against a panel of appropriate rodent antigens. Agglutinations were performed by

mixing equal portions of antiserum dilution in saline with 2 per cent erythrocyte suspensions in saline. Tests were interpreted grossly after two hours' incubation at 37°. Ring precipitin tests for serum antibodies were performed by overlaying saline dilutions of test serum over undiluted antiserum. Gross rings of precipitation were determined at the end of two hours.

Rabbit antisera to rat and mouse erythrocytes and to rat and mouse serum revealed marked crossing of antigenicity. Indeed, titers were approximately identical with appropriate antigens from either species. No cross reactivity was noted between rat or mouse antibodies and guinea pig or hamster antigens.

DISCUSSION

These studies simply confirm and amplify the fact that the rat and the mouse are immunologically very similar. Makinodan[5] found that in order to produce antisera specific for rat red cells, the mouse must be the recipient antibody producer. Smith[9] also produced specific antiplatelet serum in the same way. Our studies of heterologous antibodies again demonstrate the marked similarity of antigens in these two rodent species. This similarity is not shared with other common rodents.

Despite the fact that rat and mouse erythrocytes contain so many similar or, indeed, identical antigens, the rat erythrocyte seems to contain a good antigen in the mouse. A single injection of rat erythrocytes regularly produced antibodies in the mouse which persisted for many weeks and which resulted in accelerated destruction of rat erythrocytes.

From our studies, one can speculate on some of the mechanisms involved in the acceptance of rat bone marrow by the irradiated mouse. The two species apparently have marked antigenic similarities, so that there is no "basic" incompatibility. Whatever the mechanism of this "basic" type of incompatibility, it was not abolished (for erythrocytes from several species, at least) by heavy irradiation. This type of incompatibility for foreign erythrocytes does not seem related to humoral substances, at least of a type that are demonstrable by conventional tests for agglutination or lysis.

The type of incompatibility to rat erythrocytes that did develop in normal mice was solely related to humoral antibody production. Reaction of the mouse to the other cell antigens of the donor bone marrow would seem logically to be of this acquired, rather than "basic," incompatibility. Therefore, during the period of immune unresponsiveness following irradi-

ation and marrow treatment of the mouse, it is easy to understand the initial growth and proliferation of the donor rat bone marrow.

A number of studies, however, have shown that recovery of antibody production mechanisms return within 4-6 weeks in the irradiated, marrow-treated mouse.[8, 9] Rejection of the rat hematopoietic tissues does not occur, and Makinodan[8] has postulated that prolonged immune tolerance to the graft occurs. Odell[8] has certainly demonstrated some type of tolerance in irradiated rats treated with a separate strain marrow. These animals have been shown to produce erythrocytes of both rat types for prolonged periods of time. However, the antigenic properties of these rat erythrocyte strains are very slight.

Certainly our observations suggest that because of the marked antigenic similarities of the mouse and rat erythrocyte immune tolerance to rat marrow might occur. This would be a more attractive hypothesis, however, if the rat erythrocyte did not also possess good antigenicity in the mouse. The alternative explanation that the prolonged acceptance of rat marrow is related to total replacement of mouse immune tissues by rat donor immune tissues[10, 11] remains a possibility.

SUMMARY

1. Labeled rat erythrocytes are destroyed in the mouse by production of anti-rat erythrocyte antibodies.

2. In irradiated, nonantibody-producing mice there is no incompatibility of rat erythrocytes, and the labeled cells circulate for prolonged periods.

3. Other rodent erythrocytes are basically incompatible in the mouse and are rapidly destroyed even by the irradiated mouse recipient.

4. The similarity of antigenic structure of rat and mouse erythrocytes is manifested by marked cross-reactivity of antisera produced in the rabbit. However, rat erythrocytes regularly produced an immune response in mice.

5. The implications of these findings in relationship to the acceptance of heterologous bone marrow grafts are discussed.

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The authors would like to express their appreciation to Dr. Charles C. Congdon, Oak Ridge National Laboratory, Oak Ridge, Tennessee, for his help in initiating these studies.

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The Board of Editors

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ELECTRON MICROSCOPIC STUDIES ON THE ORGANIC MATRIX OF ENAMEL AND DENTIN

ENAMEL

In previously published electron microscopic studies on the organic substance of enamel, the organic matrix was depicted in thin sections as a meshwork of delicate fibrils.[1,2,9,12] If one compares these structures with photographs obtained by the replica method using mineralized enamel, the original relationship between the organic and the inorganic components of the enamel is difficult to reconstruct. In the decalcified thin section one sees the organic meshwork with interlacing fibrils running in all directions in a disorderly fashion. In the replica method, in contrast, the rod-like crystals of hydroxyapatite are regularly oriented within each prism. The crystals do not fit into the meshes of the fibrillar lacework. Apparently, the method of decalcification accounts for changes in the organic structures which do not permit one to consider these meshworks as the true image of the organic substance.

Other structures occurring in enamel have likewise been subjected to widely differing interpretations in electron microscopic studies. Several authors[2,12] have described an interprismatic substance (confirming, in a way, findings obtained by light microscopy), whereas others[1,4,5] have denied the existence of an interprismatic substance.

In an attempt to clarify these problems the present study has been undertaken, using toothbuds. We expected to obtain information on the structure of enamel more easily from pre-enamel, young enamel, and from the ameloblast layer, since it is possible to prepare thin sections of these structures without decalcification.

It has been previously assumed that there is an intercellular substance between the individual ameloblasts from which the interprismatic substance supposedly develops, the ameloblasts themselves giving rise to the prisms.[7–10] Our investigations have led to different conclusions. Sections

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of ameloblasts, fixed in buffered OsO_4 according to the methods that have become routine in electron microscopy, show clearly that the ameloblasts are in immediate proximity to each other, without any intervening intercellular substance (Figs. 1 and 2).** The ameloblast layer is separated from the pulp by a thin membrane invisible by light microscopy. The fibers adhering to it belong to the pulp and do not extend into the ameloblast layer.

The spaces between the individual cells that are normally seen under the light microscope are found in the electron microscopic preparations only if fixation in formalin has led to protoplasmic changes. This suggests that these structures, previously interpreted as intercellular substance, are artifacts (Fig. 3). The intercellular spaces do not occur when the tissue is fresh and well fixed.

After the dentin anlage has been formed and has begun to calcify, the ameloblasts produce the organic matrix of the primordial enamel (Figs. 4 and 5).

During this process the ameloblasts undergo a certain change that affects their protoplasm. The cell borders seem to disappear and in the zone of enamel formation delicate fibrils precipitate out. Whether this change involves the entire cell or merely a part of it cannot be stated at present. Further work on this point is in progress (Figs. 6 and 7).

The pre-enamel, previously thought to be homogeneous on the basis of light microscopy, shows, in the electron microscope, that the fibrils of its organic matrix are arranged in a well-defined way. The picture is in complete accordance with the arrangement of the hydroxyapatite crystals as demonstrated by electron microscopic studies. Here again no interprismatic substance is apparent (Figs. 8 and 9). We have to picture the process of mineralization in such a way that the preformed, well-oriented organic matrix exerts an orienting influence on the hydroxyapatite crystals. During the process of their formation the latter grow upon the framework of the organic matrix.

Following demineralization at neutral pH by means of Titriplex III (Merck) (chemically: Disodium salt of Ethylene-diamine-tetraacetic acid, known in the American literature under the name "Versenate" or "EDTA") it is possible to demonstrate, even in mature enamel, the above described structure of the organic matrix. Here again we find these elements well organized and in agreement with the structure of mineralized enamel, except for the absence of the previously described fibrillary meshwork (Fig. 10).

** All photographs were made from thin sections except Figure 9.

These investigations have shown the extent to which the appearance and structure of the enamel are determined by the organic matrix. A very close interrelation between organic and inorganic enamel substance must be assumed.

The results obtained by the chemists and mineralogists are thus confirmed histologically by electron microscopic methods.

DENTIN

As in enamel, the organic matrix in dentin also exerts a determining influence on the arrangement of the inorganic elements. However, since the organic substance of the dentin is organized in an entirely different way, the inorganic elements, which are different in form and size, are fitted into the organic matrix in another manner. Here again, examinations on dentin during the process of its formation yield the most information.

It has been assumed since the work of V. Korff,[8] that fibers originating in the pulp form the meshwork of collagen fibrils. The odontoblasts were thought to be important only in dentin formation; their significance was considered to be only secondary.

In contrast to this, electron micrographs show that the odontoblasts play a very decisive role.

After differentiation of the ameloblasts a cell-free zone exists near the pulp. No fibrils are as yet recognized in this region, which has been demonstrated by light microscopy after collagen staining (Fig. 11). The ameloblasts are separated from the pulp by a membrane, invisible in the light microscope, from which fibril-like processes extend toward the pulp (Fig. 12). Only after the differentiation of the odontoblasts does the formation of collagen fibers begin; their cross-striations are clearly visible. The activity of the odontoblasts is illustrated by their highly developed endoplasmic reticulum and a large number of mitochondria (Figs. 13 and 14).

Well-defined orientation can only be recognized along the future junction of enamel and dentin, that is, in the zone called "Manteldentin" by Weidenreich.[15] In this zone, the collagen fibers are oriented at right angles to the junction, whereas they lack definite orientation in the remainder of the dentin (Fig. 15).

After the organic matrix increases to a width of 5-10 m, calcification begins (Fig. 16). Here again, the process occurs along the fibrils (Fig. 17). In the demineralized preparation, also, the original orientation of

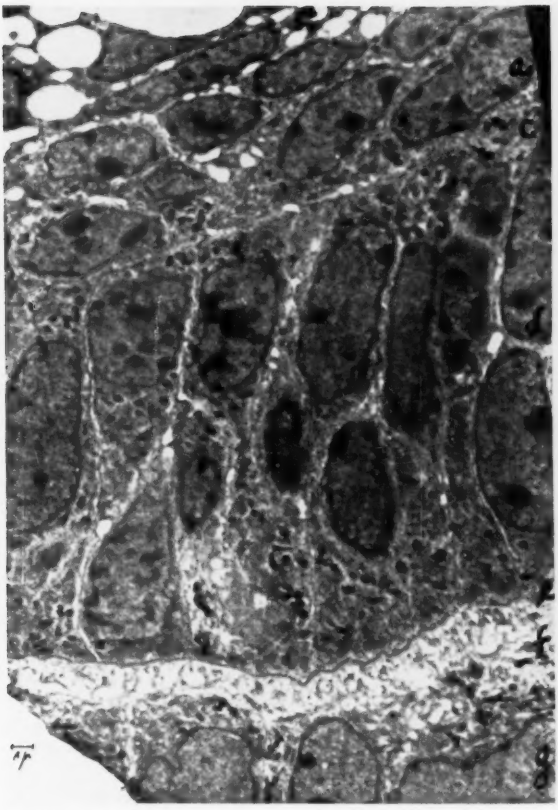

FIG. 1. Ameloblast layer with stratum intermedium (a) and enamel pulp (b); pulp with odontoblasts. Note absence of basal membrane (c). The ameloblasts (d) approach each other directly. (e = limiting membrane, f = first layer of dentin, g = odontoblasts). x3,000.

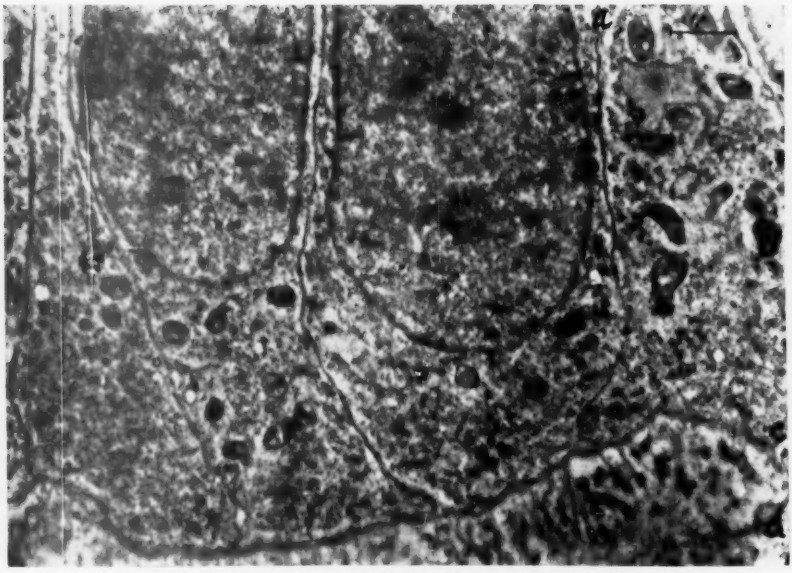

FIG. 2. Ameloblasts, fixed in OsO_4. Between the individual cells no intercellular substance can be seen (a). The cells border each other directly. A membrane separates the ameloblast layer from the pulp; b = mitochondria, c = limiting membrane, d = odontoblasts. x10,800.

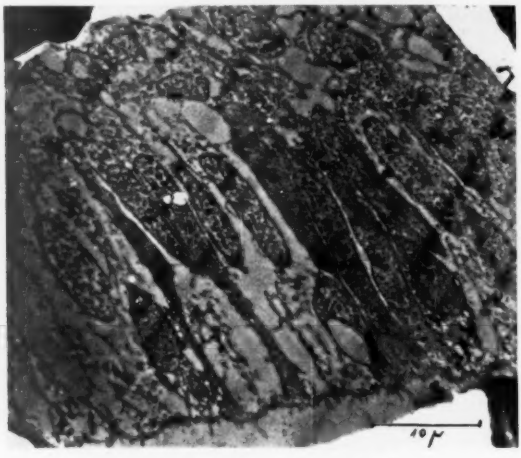

FIG. 3. Ameloblasts, fixed in formalin. In formalin-fixed material much of the protoplasm is poorly fixed, and clefts can occur in the tissue. These clefts are not intercellular substance, but artifacts. a = ameloblasts, b, the cell-free zone of the pulp. x1,680.

FIG. 4. Formation of pre-enamel, fixation in OsO_4. Precipitation of the uncalcified fibrils of the organic matrix takes place on the calcified dentin. The ameloblasts appear altered (see Figs. 1 and 2); a indicates an artifact, b, pre-enamel, and c, dentin. x1,800.

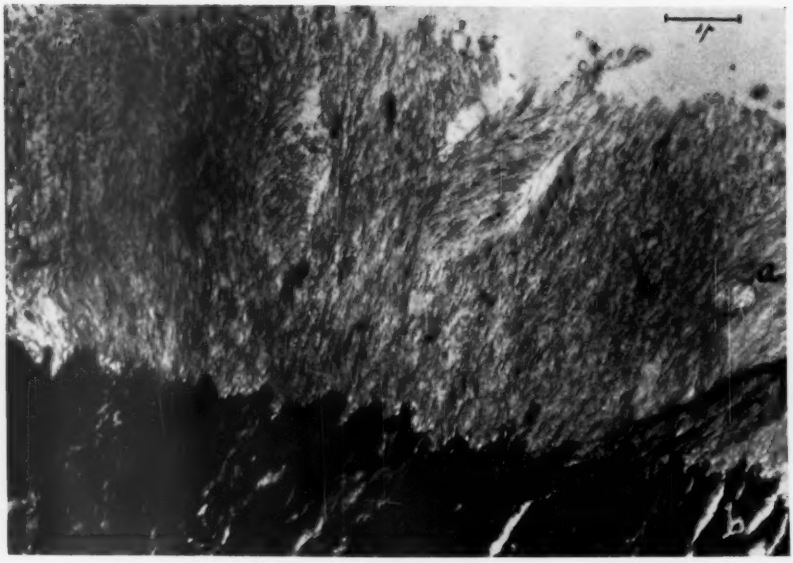

FIG. 5. Pre-enamel, fixed in OsO_4. The delicate fibrils of the organic matrix are regularly oriented. No interprismatic substance can be seen. a = pre-enamel, b, dentine. x10,800.

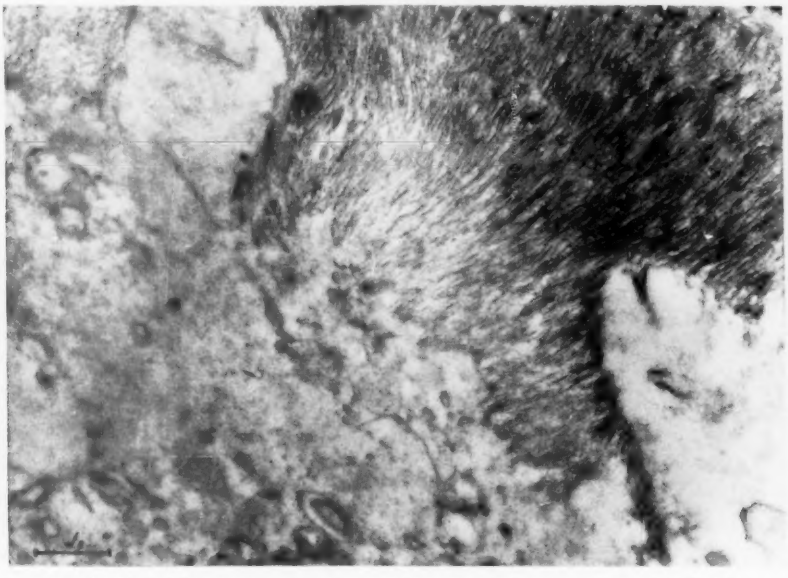

FIG. 6. Formation of the organic matrix of the enamel. Fixed in OsO_4. x10,800.

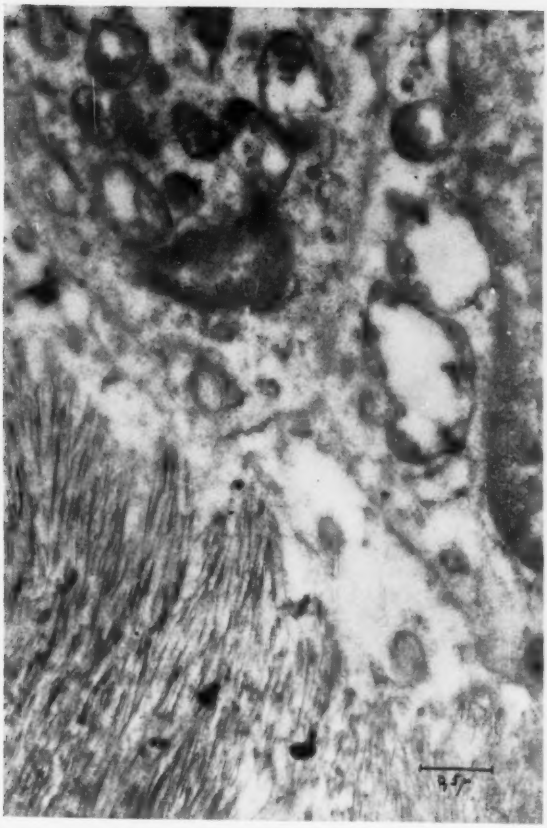

FIG. 7. Formation of the organic matrix of the enamel. Fixed in OsO_4. x22,500.

FIG. 8. Uncalcified organic matrix of pre-enamel. OsO_4 fixation. x30,000.

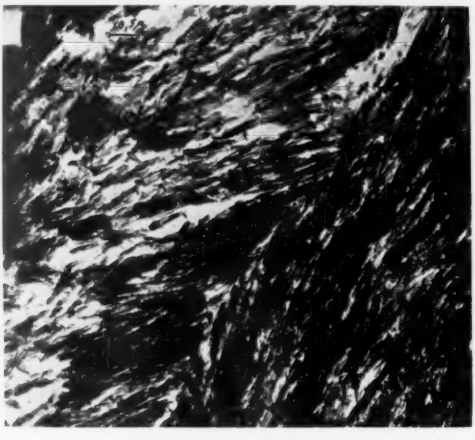

FIG. 9. Replica of normal enamel with adherent crystals of hydroxyapatite. Comparison with Figure 8 demonstrates the orienting influence exerted by the organic matrix on the crystals of the enamel. x15,000.

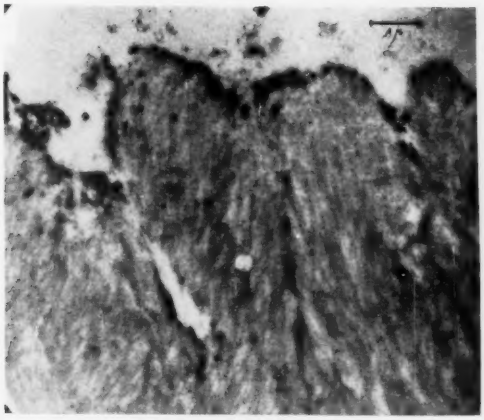

FIG. 10. Organic matrix of mature enamel, decalcified with Titriplex III and fixed in OsO_4. Here again, note that the fibrils of the organic matrix show the same arrangement as in pre-enamel. x7,200.

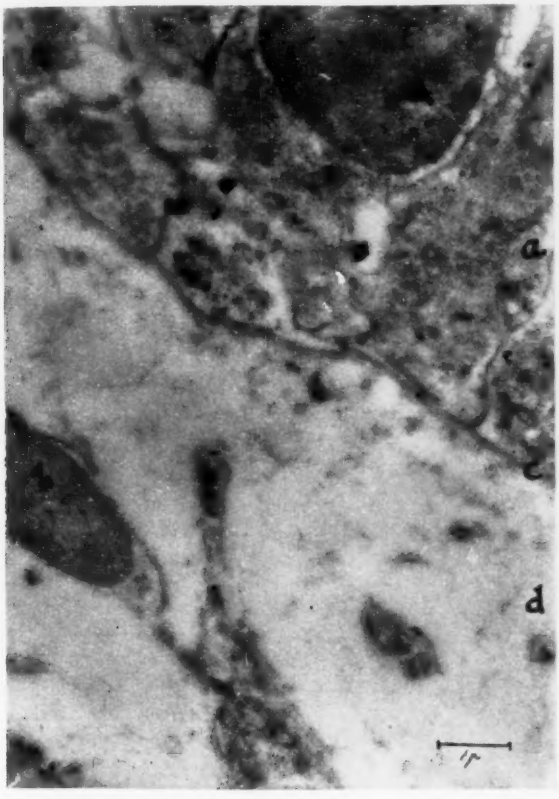

FIG. 11. Ameloblasts with the cell-free zone of the pulp, fixed in OsO_4. The differentiation of the odontoblasts has not yet taken place. Fibers originating in the pulp cannot be made out. The empty spaces in the ameloblasts layer are artifacts. a = ameloblasts, b, pulp cells, c, limiting membrane, d, cell-free zone. x10,800.

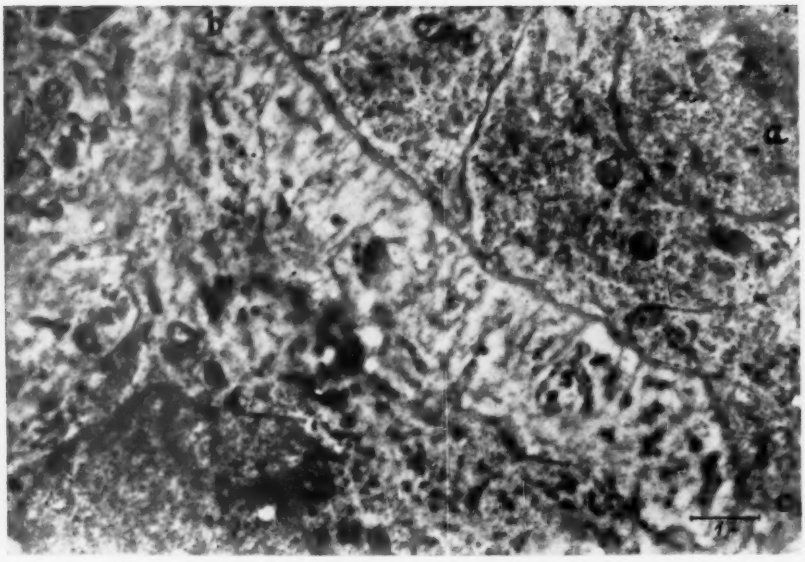

FIG. 12. Ameloblasts (a) with limiting membrane toward the pulp, fixed in OsO_4. The delicate fibrils extending from the membrane (c) are the first precursors of the organic substance of the dentin (b). x10,800.

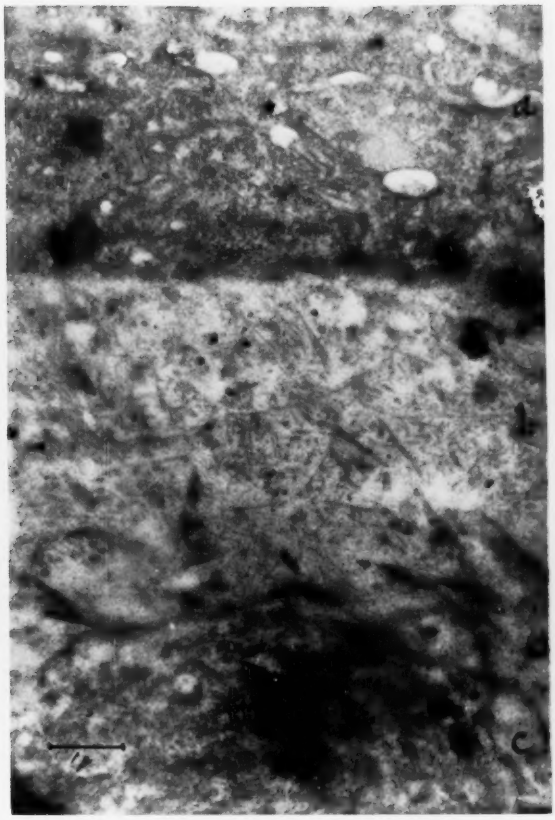

FIG. 13. Formation of the collagenous ground substance of the dentin, fixed in OsO_4. The fibrils exhibit the typical cross-striation of collagen. a = ameloblasts, b, predentine, c, odontoblasts. x10,800.

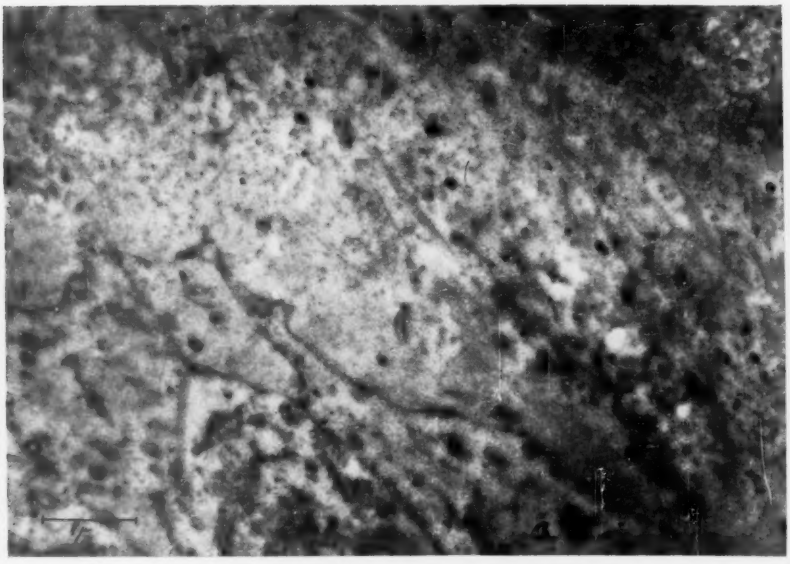

FIG. 14. Formation of the collagenous ground substance of the dentin, fixed in OsO_4; a = odontoblasts. x14,400.

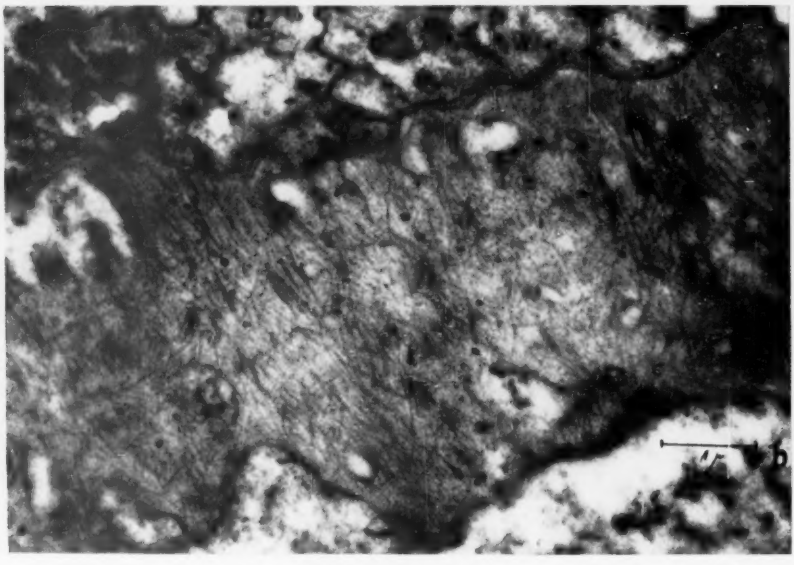

FIG. 15. Formation of the collagenous ground substance of the dentin, fixed in OsO_4. Near the future junction of enamel and dentin a regular orientation of the fibrils at right angles to the junction is noted. Towards the pulp the fibrils form a dense meshwork. a = ameloblasts, b, odontoblasts. x14,400.

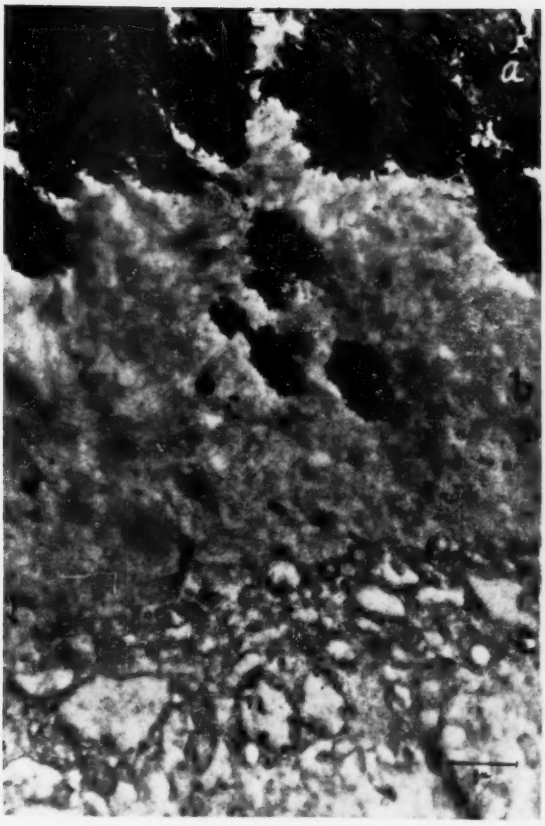

FIG. 16. Beginning mineralization of the ground substance of the dentin (a). OsO_4 fixation. The portions already mineralized are electron opaque and appear therefore black. Highly developed endoplasmic reticulum in the odontoblasts (c). Predentine is indicated by b. x10,800.

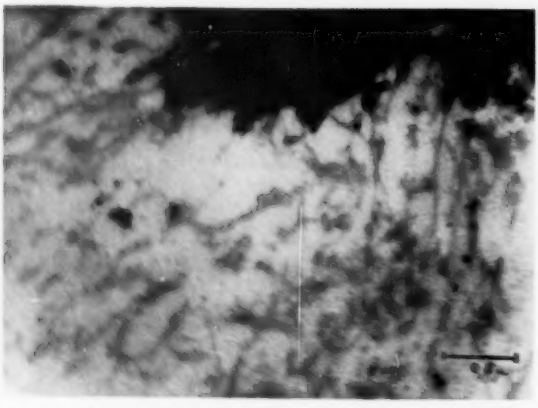

Fig. 17. The mineralization of the ground substance of the dentin occurs along the collagen fibrils. UNA fixation. ×22,800.

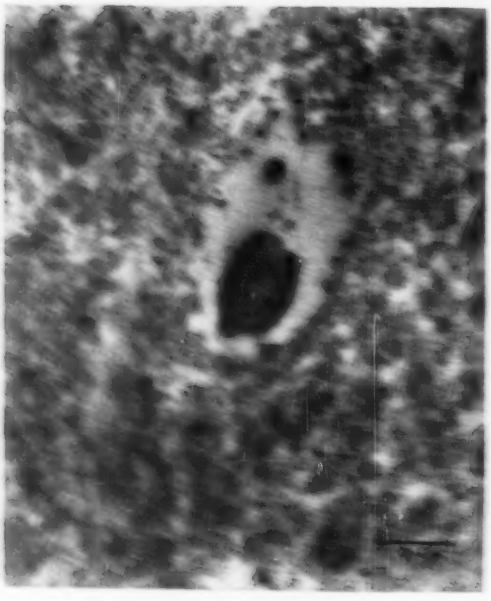

Fig. 18. Mouse molar, treatment with Formula III, over a year. The mineralization of the ground substance can be recognized even more. ×4,750.

the collagen fibers can be demonstrated (Fig. 18). The predominantly parallel orientation of the fibers inferred from polarized light studies is not confirmed by electron microscopic studies.

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EVENTS

DEPARTMENT OF ANATOMY

November 12, 1958

THE SURGICAL ANATOMY OF THE PANCREAS. By Russell T. Woodburne, Professor of Anatomy and Chairman of the Department of Anatomy, The University of Michigan Medical School, Ann Arbor.

The close relationship between surgery and anatomy is never more clearly expressed than in the development of new or more extensive surgical procedures. Current broadened surgical attacks on the pancreas will become proportionately less hazardous as the relationships of the organ and its rich blood supply and drainage are better understood. Posteriorly the gland is related to many large blood vessels: the splenic artery, the splenic vein, the renal arteries and veins, the aorta, and the inferior vena cava. Just medial to the duodenojejunal flexure the splenic vein receives the inferior mesenteric vein and it then combines with the superior mesenteric vein to form the portal vein. The large portal vein ascends against the dorsal surface of the neck of the pancreas. The superior mesenteric artery, arising from the aorta just above the renal arteries, descends behind the neck of the pancreas, and then overlies the uncinate process as it enters the mesentery.

The arteries of the gland are numerous and form a rich inter-connection between the celiac and superior mesenteric arteries. They lie largely on the dorsal surface of the gland and only a few are visible from in front. Nevertheless complete anastomosing arcades of vessels are formed in relation to the head of the pancreas. The gastroduodenal artery divides over the head of the pancreas into the right gastro-epiploic and the anterior superior pancreaticoduodenal arteries. The latter descends near the duodenal groove and connects with the anterior inferior pancreticoduodenal artery. A posterior superior pancreaticoduodenal artery, arising from the first 2 cm. of the gastroduodenal artery, makes a similar arcade on the dorsum of the head of the pancreas with the posterior inferior pancreaticoduodenal artery. The inferior pancreaticoduodenal arteries usually spring from a common trunk from the superior mesenteric or upper jejunal arteries but may exhibit separate origins from these source vessels. A dorsal pancreatic artery descends posteriorly against the neck of the pancreas and sends its left branch along the dorso-inferior border of the body of the pancreas. This is the constant inferior pancreatic artery. The right branch runs across the head of the pancreas and helps form a prepancreatic arcade. The dorsal pancreatic artery has a variety of sources: splenic, celiac, superior mesenteric, and hepatic arteries. The pancreatica magna artery and caudal pancreatic arteries, along with the inferior pancreatic artery, vascularize the body and tail of the pancreas. The venous drainage of the pancreas corresponds in general pattern to the arterial supply, but the veins are customarily anterior and to the right of the arteries. The posterior superior pancreaticoduodenal artery and vein closely spiral the common bile duct on the dorsum of the pancreas.

R. T. W.

BIOPHYSICS SEMINAR

November 17, 1958

RIBONUCLEOPROTEIN PARTICLES FROM E. COLI. By James Watson, Harvard University.

RNA forms about 25 per cent of the dry weight of *E. coli;* of this, 10 per cent is low MW "soluble RNA," and 90 per cent occurs in high MW ribonucleoprotein particles. These particles are about 60 per cent RNA; thus the RNP particles make up about 40 per cent of the cells' dry weight.

The RNP particles can be got in four sizes: (i) an irregularly shaped particle sedimenting at 32 S (MW 0.9 x 10^6), (ii) a sphere of 51 S (MW 1.8 x 10^6), (iii) a larger sphere of 70 S (MW 2.7 x 10^6), and (iv) a bispherical particle of 100 S (MW 5.4 x 10^6). The relative proportions of these different sizes depends on the Mg++ concentration. Only the 32 S and 51 S particles are found at low [Mg++]; these combine to form the 70 S at higher concentrations. The 70 S in turn dimerizes into the 100 S particle.

All the particles are electrophoretically mobile and RNAase resistant, suggesting that the protein forms an outer coat over the RNA. The protein is basic, and end group analysis suggests that it is made up of four molecular species. It reacts with formaldehyde, indicating a lack of hydrogen bonding.

If the bacteria are grown in the presence of chloramphenicol (which inhibits protein but not nucleic acid synthesis), there is a vast reduction in the number of the above four particles accompanied by the appearance of new smaller particles. The smaller particles are 75 per cent RNA, and are RNAase sensitive. At low [Mg++] a 15 S particle is found, which combines into 24 S and 31 S particles at higher concentrations. By comparison of RNP fractions got by growing cells on P^{32} both before and after chloramphenicol treatment, it was found that most but not all of the smaller particles are newly synthesized, rather than breakdown products of the normal particles. Chloramphenicol grown cells also show an increase in the soluble RNA fraction.

The evidence cited points to a considerable similarity between the bacterial RNP particles and the microsomes of other organisms.

F. M. W.

ARTHRITIS STUDY UNIT

November 20, 1958

THE PHYSICAL CHEMISTRY OF ANTIGEN-ANTIBODY REACTIONS. By Dr. S. J. Singer, Associate Professor of Chemistry, Yale University.

The number of sites involved in antigen-antibody bonding, the forces and chemical groups active, and the bond strengths of various combinations were investigated, using rabbit antibodies against proteins combined with the respective antigens in solution.

It is well known that when antigen is added to a mixture of antibody and antigen at their "equivalence" point (i.e., where all the antigen has precipitated with all the antibody), the precipitate dissolves. The reason for this is that at high concentrations of antigen the species, G-Ab-G (where G represents antigen and Ab, antibody) with a ratio of 2 G : 1 Ab and the species, G-Ab-G-Ab-G, which has a 3:2 ratio of antigen to antibody, are favored over larger aggregates in which the ratio of G:Ab is smaller. Fragmentation of the precipitate with the formation of such small species occurs upon addition of the antigen, as determined by ultra-centrifugation patterns. On the other hand, in the zone of antibody excess, such forms as:

$$\begin{array}{c} \text{Ab} \\ \text{Ab-G-Ab} \\ \text{Ab} \end{array}$$

predominate and are of such a chemical nature and large size that they too are insoluble.

Bound antigen can be calculated by subtracting free antigen from total antigen, both of which can be determined by means of ultracentrifugation or electrophoresis. Plotting bound G/Ab versus the per cent of total antigen in a solution gives a curve which may be extrapolated to 100 per cent total antigen (infinite antigen excess), at which point the bound G/Ab ratio is found to be 2:1. In other words, rabbit precipitating antibodies are divalent. Any deviation from this ratio of 2:1 in solutions at less than infinite antigen excess is an indication of the weakness of the bonding strength.

A more precise study of binding energies may be undertaken by determining the free energy change (ΔF°) of the reaction, recalling that $\Delta F^\circ = - RT \ln K$, where R is the universal gas constant, T is the temperature in Absolute units, and K is the equilibrium constant of the reaction, which may be determined experimentally. Such work has shown that the total binding energy of a protein antigen is not significantly different from that of a hapten, with their respective antibodies. Bond energies for all antigen-antibody combinations are uniformly small, which would satisfy the requirements of a template theory, where the antibody must separate from the determining antigen after formation.

Dissociation of Ab-G combinations occurs at acid and at alkaline pH's. Perhaps in one molecule's reactive sites, one or more carboxyl groups in the charged state is needed to interact with oppositely charged groups in the complementary sites for bond stability to be maximal. Experimental results on the variation of K with pH at acid pH's are consistent with this concept. At alkaline pH's an intramolecular configurational change in the antibody molecule may be involved, causing a dissociation. Intermolecular electrostatic forces are probably not significantly involved in many *Ab-G* combinations, because the magnitude of K shows no correlation with the product of the net charges on the antigen and antibody molecules.

M. S. M.

YALE MEDICAL SOCIETY

December 5, 1958

PERIPHERAL CIRCULATION. By Nicholas M. Greene, Departments of Surgery and Pharmacology.

My colleagues and I have undertaken a project to show the precise way in which anaesthetic drugs alter the oxygen requirement, peripheral circulation, and respiration of the animal or individual to whom the drug is given. As preparation for our work we had to consider the complex interrelationships of blood flow, blood pressure, and oxygen tension in post-arterial peripheral circulation. Post-arterial circulation varies in its characteristics, depending upon the organ or part of the body one studies; but if we use the circulation in the skin as our prototype, our system will have the following components: an arteriole, a metarteriole, and arteriovenous shunt extending from the metarteriole to the venule, several capillaries also extending from the metarteriole to the venule, a venule, and various sphincters which can occlude the capillaries at their metarteriolar end or diminish the diameter of the metarteriole. Depending upon the spontaneous vasomotion of the system the precapillary sphincters may operate whereby either a few or a great many red cells pass through the capillaries, and the rest of the cells will pass directly into the venule through the arteriovenous shunt. If the preponderance of the circulation is through the shunt, the oxygen tension in the venous end of the system obviously does not reflect with accuracy the utilization of oxygen by the tissue. Furthermore, the pressure and flow relationships of the system will change, depending upon the vasomotion of each or all of the components of the system. For example, the pressure in the capillary will fall if either the metarteriolar pressure is reduced due to constriction of the metarteriole (as seen in Reynaud's disease), or if the venous backpressure is increased. Conversely, vasodilatation and increased blood flow (seen as hyperemia) may arise from exercise, heat, trauma, or vasodilatory drugs, such as histamine. Since the tissue oxygen tension is related to the rate of blood flow, when the velocity of the blood rises and oxygen utilization is unchanged, the oxygen tension at the venous end of the system will rise; conversely, tissue oxygen tension will fall with a decrease in velocity though oxygen utilization is unchanged. Other assumptions further utilized the equation (Ohm's law) that Flow = Pressure/Resistance, so we assumed that with a constant arteriolar pressure an increased resistance would produce a decline in flow, and a decreased resistance would produce a greater flow.

The studies I wish to report on today were to determine the effects of sympathetic denervation on skin oxygen consumption in patients. Our measurements involved the use of a platinum electrode of fixed voltage; the electrode reduced any molecular oxygen present, and a galvanometric deflection indicated directly the amount of oxygen reduced and therefore originally present. Some of our data were as follows: (i) During a period of "denitrogenation" of a nonsympathectomized patient (during which the patient breathed only oxygen), an increase of 300 per cent in the skin tension was seen (ii) with a temporary sympathetic block (spinal anesthesia or para-

vertebral block) skin oxygen tension may fall precipitously or only slightly. In interpreting our results it was concluded that oxygen tension of the skin during sympathetic denervation was related solely to the oxygen supplied the tissue. Oxygen supply depends upon three factors: the coefficient of oxygen diffusion, the oxygen tension in the entering arterial blood, and the rate at which the oxygen is brought to the tissue by capillary blood flow. Only the latter factor is altered by sympathetic block. The results suggest that criteria of the adequacy of capillary blood flow based upon either skin temperature determinations or blood flow in terms of cc. (blood)/100 gm. (tissue) /minute may be misleading under certain circumstances because they do not take into account the fact that in the hyperemic state the velocity of capillary blood flow may become inadequate to maintain normal tissue oxygen tensions.

In studying the physiological significance of changes in tissue oxygen tensions, biochemical indices such as changes in serum lactate, pyruvate, and potassium may be used. Care must, however, be taken to differentiate the metabolic response due to reflex epinephrine release occasioned by the hypoxia from the metabolic response to hypoxia per se. In experimental animals, data were obtained indicating that about one-third of the metabolic response to hypoxia may be ascribed to the release of epinephrine and not to the hypoxia itself. Preliminary data in patients with a complete sympathetic block indicate that the decreases in skin oxygen tension observed during limited sympathetic denervation are not associated with significant metabolic evidences of hypoxia.

H. J. L.

BOOK REVIEWS

EVOLUTION BY NATURAL SELECTION: *A Centenary Commemorative Volume.* Papers by Charles Darwin and Alfred Russel Wallace. New York, Cambridge University Press, 1958. 287 pp. $4.75.

The first published expression of the theory of evolution by natural selection was printed July 1, 1858, in the Journal of the Linnaean Society of London in the form of two essays: one by Charles Darwin and one by Alfred Russel Wallace. These two essays, along with Darwin's preliminary *Sketch* of 1842 and his *Essay* of 1844 (neither of which were published until 1909), and a foreword by Sir Gavin de Beer have been brought together in this commemorative centenary volume.

The *Sketch* of 1842 is by no means a polished essay; in fact, as Sir Francis Darwin points out in his introduction, it "is more like hasty memoranda of what was clear to himself, than material for the convincing of others." The *Essay* of 1844 is an expansion of the *Sketch* written from memory and apparently was not intended for publication. As little known precursors of the Darwin-Wallace Essays of 1858, the *Sketch* of 1842 and the *Essay* of 1844 make fascinating reading.

This year has seen a spate of magazine articles, scholarly essays, and books celebrating the hundredth birthday of the first publication of the theory of evolution by natural selection; more than likely 1959 will also be a bumper year, commemorating the centenary of the *Origin of Species.* Many of these writings have been preoccupied with the development of Darwin's ideas on the origin of species. It is refreshing to be able to read Darwin's own words on this subject and to have them put into such clear perspective by Sir Gavin de Beer in his foreword to this particularly appropriate commemorative volume.

PHILIP S. HUMPHREY

BACTERIAL AND MYCOTIC INFECTIONS OF MAN. 3d Edition. Edited by Rene J. Dubos. Philadelphia, J. B. Lippincott Co., 1958. 820 pp. $8.50.

This is the third edition of an already well-accepted text which covers the field of infectious disease very adequately. There is no doubt that this will continue to be as popular as the last edition which came out six years ago, and it should be appealing to all those who have an interest in medical microbiology. Even the clinician will be pleased with the authors' approach. The material is up to date, and Dr. Dubos and his group of experts, some of whom are new contributors, are to be given credit for their efforts.

Two new chapters are included. Chapter 2 on "The Evolution and Ecology of Microbial Diseases" by Dr. Dubos is admittedly controversial and speculative. He deals with the host-parasite relationship and stresses the point that genetic and immunologic factors and evolutionary adaptation among other things dictate that we must deal differently with the same relationship depending on the time and place it happens to occur. He presents the intriguing thought that bacteria are capable of peaceful coexistence with their host, and that if there is a difference between infec-

tion and overt disease, we should study the factors which influence the conversion of the former to the latter.

The second new chapter is Chapter 34 on "Chemotherapy of Microbial Diseases" by Dr. Walsh McDermott, who has done extensive work with the tubercle bacillus in recent years. This and the preceding chapter on chemotherapeutic principles are really a division of a single chapter in the previous edition, but the division is a logical one and makes for better organization. His discussion of *in vivo* drug-parasite relationships has been expanded, and the section on resistance as related to multiple drug therapy is especially timely.

Many chapters have been revised extensively, but several are essentially the same. The chapter on serology and immunochemistry has been expanded and includes such novel concepts as immune adherence and the properidin system. The chapter on bacterial morphology, physiology, and genetics has been changed appreciably too, especially with the inclusion of new material on the mutation of bacteria.

The book itself is an attractive one; the paper is high grade and the illustrations are well done. The bibliography has been cut down (and this is perhaps the only factor not in favor of this edition) as well as the size of the print to keep the size of the volume to a minimum. Also, thanks to The National Foundation we are able to purchase *Bacterial and Mycotic Infections of Man* for an attractive price—a rare happening these days.

J. A. O'N.

HUMAN HISTOLOGY. By Leslie Brainerd Arey. Philadelphia and London, W. B. Saunders Co., 1957. ix, 337 pp. $6.50.

The author of this textbook in outline form is an established authority in the field of histology. To quote him, "It is a full-length textbook, standard in scope, unorthodox in treatment and designedly without illustrations." The material is presented clearly with the information arranged under heads and subheads. The concise text consists mostly of single line sentences embodying an outline treatment by both topic headings and indented descriptions with the liberal and consistent use of italicized terms and phrases to catch the eye and add emphasis. There is a brief introduction to each tissue and organ to serve as a quick preview of the main organizational plan followed by an extended treatment of their detailed structure. A practical summary of each tissue as it appears in sections and the diagnostic features of each organ is included to offer aids to interpretation and diagnosis. Functional correlations are presented to point out the essential relations of function to the known structural basis of a tissue or organ, and their regenerative capacities are summarized.

The student studying histology for the first time probably would not find that this book replaces the standard histological textbooks which contain extensive descriptive discussions correlated with accompanying illustrative material. However, he would find it to be a valuable companion to laboratory study and a quick review source of the essentials of the topic he is studying.

Teachers and research investigators would find the book to be exceptionally useful as a reference source when the need arose to check quickly a specific point concerning a tissue or organ on the histological level.

E. S. CRELIN

TUBERCULOSIS IN WHITE AND NEGRO CHILDREN. Vol. II, The Epidemiologic Aspects of the Harriet Lane Study. By M. E. Brailey. Published for the Commonwealth Fund by the Harvard University Press, Cambridge, Massachusetts, 1958. 103 pp. $4.50.

The Harriet Lane Study was carried out over the period between 1928 and 1950, in the era before specific antibiotics and chemotherapy against tuberculosis became available. The second volume describing the results deals with epidemological aspects; it provides a carefully documented picture of the natural history of the disease in childhood, a valuable contribution which cannot be duplicated in the present chemotherapeutic age.

The objectives of the study are centered around the factors altering the prognosis of tuberculosis infection in childhood, i.e.: What per cent of infected children develop clinical disease, and how many die of it? How do such factors as race, sex, age at which the infection is acquired, and environment, alter the prognosis? The study group consisted of 1,329 children, 437 white and 892 Negroes, all of whom came from a similar low socio-economic environment. Patients were drawn from the Harriet Lane Tuberculosis Clinic, which admitted only children under two years of age who were already tuberculin positive; in addition, their older siblings up to age 15, who were found to be infected as evidenced by a positive tuberculin test, were included. Periodic x-rays, physical examinations, and home visits carried out over a 15-year period provided the data for analysis.

Although the study was carried out at a time when mortality from tuberculosis was declining sharply in the Baltimore area as elsewhere, the results indicate that the hazards of childhood tuberculosis infection were not inconsiderable. The increased susceptibility of Negro children to serious disease and their higher mortality rates were well illustrated. The highest incidence of serious pulmonary lesions on initial examination proved to be in children under one year of age in both races. Although there was no striking difference in morbidity in whites and Negroes, miliary tuberculosis was approximately twice as common in Negroes in this youngest age group. Mortality over the 15-year period considered by age at which infection was acquired was 9 per cent for whites and 22 per cent for Negroes in those infected before age 3; for those infected between 3 and 15 years, it was 0.8 per cent and 8 per cent for white and Negro children respectively. No significant differences in mortality were observed between males and females.

The same differences between whites and Negroes came out in terms of re-infection tuberculosis in children under 15. Thus, 2 of 434 white children (0.034 per cent) and 32 of 858 Negroes (0.33 per cent) developed re-infection pulmonary tuberculosis during the course of the study. Part

of this higher incidence among Negro children seems to be related to greater household exposure to sputum positive adults. In any event, the opinion that superinfection is harmless for infected children was shown not true for Negroes, who with their greater susceptibility, tend to develop progressive pulmonary tuberculosis before adolescence, as well as showing a sharp increase in incidence in the teens and early twenties.

Throughout the book, the data—extensive and complex as they are—are presented in lucid and readable fashion, aided by excellent tables and graphs. There is an excellent review of the literature. The study extends our knowledge of childhood tuberculosis and clarifies many features which remained unclear before. The volume is an important addition to tuberculosis literature.

DOROTHY M. HORSTMANN

A Doctor Speaks His Mind. By Roger I. Lee. Boston, Little, Brown & Co., 1958. 120 pp. $3.00.

The author of these essays bases his conclusions concerning the colossus of medicine on a life rich in the experience of over fifty years of medical practice. Dr. Lee was graduated from the Harvard Medical School in the first decade of this century and interned at the Massachusetts General Hospital. In addition to his medical practice, he has served as Chairman of the Board of Trustees of the American Medical Association, President of the American College of Physicians, Professor of Hygiene at Harvard, a member of the Harvard Corporation, and acting Dean of the Harvard School of Public Health.

In this compact collection of essays, Dr. Lee presents his views on many of the characteristics and features of the medical world. Among the topics discussed in the nineteen essays are the general practitioner, medical specialists, the doctor's vacation, Blue Cross and Blue Shield, public health, medical patents, medical progress, and health examinations. This reviewer, having a particular interest in public health, rather enjoyed Dr. Lee's discussion of this profession and the misunderstandings which frequently arise between it and the medical profession. He summarizes much of the problem in these two sentences. "Public Health was the legitimate and planned child of the medical profession. But parents often find their children difficult even as children usually find their parents difficult."

These "musings . . . mostly mundane but related to medical matters . . . are . . . mild but at times militant in defense of the medical profession. . ." They offer delightful and refreshing pastime for those all too few leisure periods which practitioners and medical students are able to find.

WILLIAM L. KISSICK

Rehabilitation: A Community Challenge. By W. Scott Allan. New York, J. Wiley & Sons, 1958. 247 pp. $5.75.

This book meets exactly the need which it intends to fulfill. It is an informative, thought-provoking, and well-written book on the timely and pertinent subject of rehabilitation—defined as "restoration of the handi-

capped to the fullest physical, mental, social, vocational usefulness of which they are capable." The book is aimed at the general reader as well as the student and professional worker in the field of rehabilitation and is primarily meant as a review of the field of rehabilitation rather than a "how-to-do-it manual" of detailed scientific and technical information. With this purpose in mind the author has very ably produced a comprehensive if not all-conclusive review of his subject matter and has also introduced the need for and the development of new concepts in the field of rehabilitation. The latter implied purpose of Mr. Allan's book is and should be a challenge to the reader in its discussion of the very basic and inherent philosophy of the rehabilitation field and should stimulate individual and community thought and action toward more realistic and effective rehabilitation services.

Mr. Allan summarizes the development of rehabilitation concepts and philosophy, describes facilities, services, and various professional disciplines engaged in rehabilitation work, discusses social legislation and health insurances and their effect on rehabilitation, and finally stimulates further thought and thus, hopefully, action toward more effective rehabilitation programs on local, national, and international levels by application of the basic concepts and principles of rehabilitation to organized efforts on the part of communities. Rehabilitation is not in the province of any one profession or group but rather is the responsibility of the community—a community challenge.

The following points of emphasis were made in Mr. Allan's book. Rehabilitation needs to be a cooperative endeavor, in which emphasis must be placed on coordination and integration of specialties and services to achieve uniformity and unanimity in approach to a common goal of restoration. In our age of specialization, the patient as a whole may be forgotten in professional concentration upon applying specialized services in a limited sense. We must not lose sight of the person in need of these specialized services if we are to avoid viewing merely the medical, social, psychological, and vocational problems. No disabled person is less than the sum total of his needs.

There is further need to incorporate this thinking in the medical specialties. With the advances and the discoveries in the medical sciences in recent decades, a shift of emphasis from the prime concentration upon the curative or acute treatment phase of medical care to greater interest in treating the effects of trauma, congenital conditions, and chronic and degenerative diseases has taken place.

Only in the past few years recognition is given by increasing numbers of people to the fact that it is more advantageous socially and economically to restore a disabled individual to a responsible role in society than to pay him indefinitely for his disability. This trend of thinking and practice will necessitate expansion of rehabilitation services to make them available to every handicapped person. Today there are still vast gaps between rehabilitation concepts and practices.

RENATE DORPALEN

MORPHOGENESIS: THE ANALYSIS OF MOLLUSCAN DEVELOPMENT. By Chr. P. Raven. New York, Pergamon Press, 1958. xii, 311 pp. $10.

More facts are known about the embryology of the Mollusca than about that of any other invertebrate phylum, except perhaps the Echinodermata. Investigators of the stature of E. G. Conklin, E. B. Wilson, and T. H. Morgan worked intensively on Molluscan ontogeny, and in conjunction with other workers, laid the foundation for much of what is known today. It seems particularly appropriate that this field should be summarized at the present time for several reasons; first, many new facts have become available, especially from cytological and cytochemical studies; and second, much of this newly available evidence suggests that a re-interpretation of long accepted relationships is in order.

Professor Raven is eminently qualified to undertake this summarization and synthesis. The book that he has produced is surely the most complete review of facts about Molluscan embryos that is now available. The subjects treated are Oogenesis, Maturation and Fertilization, Cleavage, Gastrulation, Embryogenesis, and Organogenesis. Chapters are divided into numerous sections covering aspects of each over-all topic and many of the sections are in turn subdivided, often to the extent that single paragraphs stand alone (as those dealing with specific cytochemical properties). This treatment will be particularly useful to the reader who employs the book as a source for references or for information on specific points. Unfortunately, it seriously detracts from continuity and makes thorough reading an onerous task. A portion of each chapter, except the first, is devoted to a "Causal Analysis" of that particular subject. In general these sections of the book will be the focal point for nonspecialists in Molluscan development.

It is while discussing the causal mechanisms of development that Raven theorizes and introduces his own interpretation of sundry phenomena. In particular, one might cite the causal analysis section of Embryogenesis; here the author marshals much modern evidence that supports the view that all early blastomeres are not strictly determined, and that the hypothesis of mosaic development may no longer be tenable. Instead, it seems probable that certain morphogenetic substances (still undefined) are localized during the cleavage process, and as a result, "chemo-differentiation" occurs. By such a process combinations of cytoplasmic substances become segregated so that a specific histo-chemical equilibrium exists in every embryonic area. Typical harmonic development is dependent upon the maintenance or normal ontogenetic change of such equilibria.

The final chapter in the book, entitled "Conclusions," is a lucid survey of Molluscan embryology as it stands today. The author believes that two major ideas can be extracted from the book: (i) that the classical idea of mosaic development must be replaced by one of dynamic change, and (ii), that the ontogeny of molluscs is basically similar to that of most other animal groups.

The book is recommended to all those interested in the specificities of invertebrate embryology, but not to those desiring a general or comparative text. Because many chapters are subdivided to the point where the book

becomes dictionary-like, and because theoretical discussion is limited to the end of each chapter, large parts of the book might be expected to be quite dull to a student reader; therefore, it cannot be recommended as a text. For these same reasons, as previously mentioned, and because of the outstanding documentation and bibliography, the book is better adapted to the needs of the more advanced researcher or lecturer. I am afraid, however, that even he will find the book to be tedious reading and will prefer to use it as a source book for specific information.

The Author, Taxonomic, and Subject Indexes are quite complete and should be of value. The photographs and drawings are of excellent quality and are admirably chosen to illustrate important points. The binding seems to be adequate at most, and does not reflect the relatively high price of the volume.

N. K. WESSELLS

LOOKING AT CHROMOSOMES. By John McLeish and Brian Snoad. New York, St. Martin's Press, 1958. 88 pp. $3.75.

The expressed aim of this attractive little book is to provide a very general introduction to chromosome structure and behavior, particularly in relation to Mendelian inheritance. The book is intended for readers who already possess a general knowledge of biology.

The short textual treatment (25 pages) is organized around a closely seriated group of very fine photomicrographs of mitosis and male and female meiosis in a flowering plant, *Lilium regale*. In spite of this brevity, the authors succeed in lucidly presenting a considerable amount of information.

Naturally so synoptic a treatment of this field is certain to produce some oversimplification, but McLeish and Snoad generally manage to avoid misleading abridgment. However, their discussion of spindle structure and formation has been abbreviated at the expense of accuracy. Furthermore, the treatment of such controversial topics as the time of chromosome and kinetochore doubling in meiosis as compared with mitosis, and the relation between chiasmata and crossing-over could have been less doctrinal with no substantial increase in length, or simply omitted.

In spite of these reservations, the book can be recommended highly to those in the medical profession and to educated laymen who wish to acquaint themselves almost painlessly with some basic and important facts about chromosomes and their relation to genetics. It may be pointed out here that all the general facts and discussions are as applicable to animals as to plants. This book will also be useful in the classroom as collateral reading during the early stages of study in cytology and genetics.

R. BRUCE NICKLAS

PSYCHOLOGICAL STRESS. By Irving L. Janis. New York, John Wiley & Sons, Inc. 1958. 439 pp. $6.95.

This volume by a member of the Yale University department of psychology reports on psychoanalytic and behavioral studies of the emotional adapta-

tions made by hospitalized patients to the stress of surgery. Detailed inquiry and observation were made of 22 patients before and after surgery; a questionnaire survey of 149 undergraduates provided retrospective accounts of their pre- and post-operative reactions. With this descriptive material, the author relates the level of pre-operative fear to post-operative behavior. Those with marked fear, which is based on pre-existing neurotic anxiety about bodily damage, develop abnormal post-operative fearfulness of injury and occasional angry outbursts. The optimistically indifferent group, who give evidence of pathological denial of the real dangers, and who avoid opportunities for preparatory information, acquire a sense of "blanket immunity" and are prone to severe disappointment. Resistant and aggressive after surgery, they impede their own recovery and pose serious hospital problems. The author proposes preventive counseling, tailored to the personality of the patient, his level of fear and type of surgery. The aim is to allay unrealistic expectations, familiarize the patient with the aspects of the hospital that touch him, promote the desirable moderate fear and the "work of worrying," and facilitate, with information, an "emotional inoculation" for surgery and post-operative suffering.

A psychological regression, with activation of dependent drives, primitive defenses like denial, magical thinking and archaic beliefs about the body, is expected with impending surgery. To explore this and other factors mediating the effects found in his phenomenological studies, the author describes the impact of surgery on a patient in psychoanalysis. Being well embarked on the regressive analytic process, the patient reported fantasied fears and expectations of surgery, and responded with infantile maneuvers to the separation from home, family, and analyst. In the last respect, the account seems incomplete—the reactions of analyst and patient to their separation, and the effect on her communications and hospital experience.

The stress of hospital surgery has its unique quality, experienced as it is in a bewildering setting, among an alien and exclusive group with arcane rituals, with certainty of bodily wounding at the hands of a person invested in advance with intense transference feelings. This study describes the patient's contribution to his reaction to the stress. It remains to complete the picture of the total context of this experience, with equivalent observations on the adaptations of the ambient nurses and surgeons and their emotional transactions with the patient, and to catalogue the complementary fantasies lived out in their work which help mediate the patient's response. More light might then be thrown on the extreme and devastating sequelae of surgery, which, while occurring in predisposed persons, are moulded by the social setting.

CHARLES GARDNER

NEUROLOGICAL BASIS OF BEHAVIOUR. Ciba Foundation Symposium. G.E.W. Wolstenholme and C. M. O'Connor, Eds. Boston, Little, Brown & Co., 1958. 400 pp., $9.00.

This book is an excellent collection of papers by leading authorities who met in London under the auspices of the Ciba Foundation to commemorate

the Sherrington centennial. The chairman, Professor Harris, mentioned in his opening remarks that anatomical, physiological, biochemical, and pharmacological study of behavior may proceed for many years "without the introduction of this somewhat abstract question of mind." In the reviewer's opinion this is certainly true, but it would be beneficial to have included in the symposium a discussion of the mind itself. In dealing with blood pressure, for example, physical or chemical investigations may be adequate, but the most important aspect of behavior escapes when only physics and chemistry are used as research tools. Behavior includes more than strict neurophysiology and it would have been useful to discuss which facets of behavior have a neurological basis and which of them elude experimental investigation.

The volume comprises a very interesting collection of nineteen papers, dealing with somewhat independent problems, which makes its review as a unit rather difficult. Discussions by several participants follow each paper, presenting fact and speculation informally, giving the book a lively quality. Actually, many of the discussions represent the most provocative and lucid thinking of the symposium. The first chapter sketches the historical ideas of mind-brain relationship (Magoun). The second chapter (Eccles) deals with neuron physiology and presents the interesting, although controversial, postulate that functionally there are just two types of nerve cells in the central nervous system—excitatory and inhibitory: all inhibitory cells are short-axon neurons lying in the gray matter, while all transmission pathways are formed by the axons of excitatory cells. Other chapters analyze the effect of brain destruction on behavior: chronically decerebate cats (Bard *et al.*), lesions of the thalamic nuclei (Brierley and Beck), and temporal lobe ablation (Klüver). Implanted electrodes and stimulation techniques are mentioned in chapters dealing with drinking induced by diencephalic stimulation in goats (Andersson *et al.*), and behavioral and electroencephalographic effects caused by electrical stimulation of reticular system in which arousal reaction, oral behavior, and motor effects may be observed (Monnier and Tissot).

A novelty in behavioral studies is the slow-release implant of sexual hormones in the hypothalamus (Harris *et al.*) which induces sexual behavior in the female cat. The study of cerebro-biochemical correlates in strains of rats selected because of behavioral differences (Rosenzweig *et al.*) marks a new and very controversial type of research, as evidenced by the sharp discussion of Dr. Klüver. "Reward" systems of the brain are discussed by Dr. Olds, who discovered them. The clinical aspects of behavior are presented by Penfield, who studied the role of temporal lobe in interpretation of present experience, and by Sherwood, who injected drugs into the ventricles of animals and humans. Conditioning, effect of drugs, problems of stress, and recording of electrical activity with microelectrodes are subjects of other chapters. Altogether, this book represents an outstanding contribution because of the material presented and the authority of the participants. Bibliographical references are in general rather short but useful to direct further reading.

JOSÉ M. R. DELGADO

HORMONE PRODUCTION IN ENDOCRINE TUMOURS. Ciba Foundation Colloquia on Endocrinology, Vol. 12. G.E.W. Wolstenholme and Maeve O'Connor, Eds. Boston, Little, Brown and Company, 1958. 351 pp. $9.00.

This volume is the twelfth in the series of Ciba Foundation Colloquia on Endocrinology, and included in this book is an index to all twelve volumes. According to the preface these published proceedings of small informal conferences are "intended for a world-wide readership, especially in regions where recent and adequately representative literature is difficult to come by," and it may fairly be said that in this new publication these aims have been amply fulfilled. The international flavor is exemplified by the origins of the participants, ranging from Bombay to Kansas.

The first two contributions deal with experimental induction of functioning pituitary tumours in mice, rats, and hamsters. Out of this work arises the interesting possibility that monomorphous pituitary tumours may one day be grown *in vitro* as a source of tropic hormones. Next Dr. Tata discourses on the interesting finding of an iodinated protein—distinct from thyroglobulin—in the plasma of nearly 60 per cent of cases of untreated thyroid carcinoma, in addition to the normally occurring thyroxine, triiodothyronine, and inorganic iodide.

Dr. Dorfman reviews the ways in which studies of human tumour slices incubated with steroids have thrown light on androgen, estrogen, and corticoid biosynthesis. A refreshing analysis of clinical adrenal abnormalities is made by Dr. Alice Robinson and her co-workers from St. Bartholomew's Hospital, London. The positive significance of grossly elevated levels of urinary 17-ketosteroids and 3β-hydroxy-17-ketosteroids in indicating an adrenal tumour is reaffirmed, as well as the fact that normal levels do not exclude it. Opinion seems to be hardening that "idiopathic hirsuties" may represent a biochemical abnormality intermediate in severity between the normal state and frank virilism. Cortisone reduces the 17-ketosteroid excretion to normal in these patients and restores menstruation, without, however, affecting the hirsuties.

A pair of clinical papers comes from Dr. Hamburger of Copenhagen, who analyses the excretion of chorionic and hypophysial gonadotropins in malignant testis tumours, and of chorionic gonadotropins in cases of hydatidiform mole and chorionepithelioma of the uterus.

Chromaffin and islet-cell tumours, as well as cysts and granulosa-cell tumours of the ovary, are all discussed, and it is clear that adequate coverage of the endocrine field has been achieved. Considering the specialised nature of the material at first sight, it is surprising how much there is of interest, if not of value, to general physicians.

DENIS ABELSON

MICROTECHNIQUES OF CLINICAL CHEMISTRY FOR THE ROUTINE LABORATORY. By Samuel Natelson. Springfield, Charles C Thomas, 1957. 484 pp. $11.00.

This book is primarily an annotated and illustrated manual of procedures employed in the author's laboratory. The coverage is rather compre-

hensive and includes such determinations as blood hematocrit, serum and urine sulfate, blood lactic acid, urine 17-ketosteroids, and the identification of sugars in the urine by means of paper chromatography. They are arranged in alphabetical order, and for each there is first a description of the procedure followed by explanatory notes (including a brief statement of the theoretical basis of the determination), instructions for making up reagents, and a bibliography.

The main portion of the text is preceded by a short illustrated discussion of the principles and of some of the specialized equipment used in microchemistry. An appendix contains a discussion of the accuracy and precision of clinical chemical methodology in general, and a description of statistical methods used in evaluating the results of analyses. The determining of the limits of normal and the comparison of data obtained under different conditions (e.g. different analytical procedures used to determine the same substance) are discussed.

Dr. Natelson's thesis is that many of the common clinical chemical analyses can be modified or substituted so that one can perform them on "micro" quantities of blood quickly, efficiently, and as routine procedures. A good portion of the methods described require considerably less than the maximum for his definition of "micro": up to 0.1 ml. In some instances the author appears to have sacrificed some degree of theoretical accuracy in order to employ such small amounts. For example, he determines the plasma alpha amino nitrogen concentration by means of a photometric method requiring 0.02 to 0.05 ml. of plasma in preference to the more specific and more accurate ninhydrin—CO_2 method requiring at least 0.5 ml. The relative ease in working with micro methods which the author stresses is also open to some question. The extra time and care involved in handling micro amounts when compared to working with almost as small quantities using the newer semi-automatic pipet-buret combinations would perhaps not be as worthwhile if the laboratory performed no determinations on small children.

The comprehensive, practical nature of the book precludes critical discussion by the author of individual determinations, and any but the briefest reference to alternate methods of analysis. Also some of the procedural details refer to specific items of equipment for which entirely adequate substitutes are likely to be available in most laboratories.

It is perhaps unnecessary to point out that the setting up of micro procedures in any laboratory is not to be lightly undertaken nor are consistent results as commonly attained as the author would imply. However, the need for such methods is rather pressing and Dr. Natelson has provided a practical, although perhaps a little too uncritical, introduction to the field.

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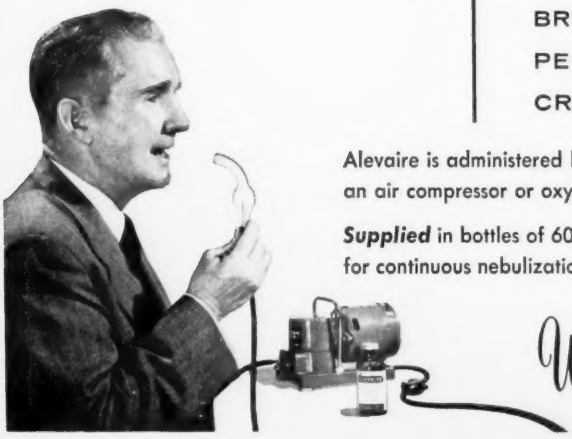

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